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Pharmacogenomics in Precision Medicine

From a Perspective of Ethnic Differences

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ISBN 978-981-15-3894-0 ISBN 978-981-15-3895-7 (eBook)
https://doi.org/10.1007/978-981-15-3895-7

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

This book focuses on the pharmacogenomics and precision medicine which is to maximize the likelihood of therapeutic efficacy and to minimize the risk of drug toxicity for an individual patient.

This book introduces the principles of pharmacogenomics and precision medicine, followed by the pharmacogenomics aspects of major therapeutic areas such as cardiovascular disease, cancer, organ transplantation, psychiatry, infection, and thrombotic disease. It also includes related areas of genotyping technology and therapeutic drug monitoring in pharmacogenomics; gut microbiota; ethical, legal, and regulatory issues; cost-effectiveness of pharmacogenomics-guided drug therapy; application of pharmacogenomics in drug discovery and development; and clinical implementation of pharmacogenomics for personalized precision medicine.

The ethnic difference of drug effect has become one of the important factors influencing drug uses, medication management, clinical trial, and development of new drugs. Therefore, we have paid much attention from a perspective of ethnic differences to pharmacokinetics and pharmacodynamics caused by genetic polymorphism in a purpose of rational use and development of existing and new medications.

The contributors of *Pharmacogenomics in Precision Medicine* come from a team of experts, including professors from academic institutions and practitioners from hospitals. This book will give an in-depth overview of the current state of pharmacogenomics in drug therapy for all health care professionals and graduate students in the ear of precision medicine.

Shanghai, China
January 2020

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Chapter 1
Introduction and Principles of Pharmacogenomics in Precision Medicine

Weimin Cai and Ziteng Wang

Abstract The individual and ethnic differences of drug effects are very important issues in clinical drug therapy. They may be caused by genetic variations which mainly come from polymorphisms of genes encoding metabolic enzymes, transporters and drug targets that affect the in vivo pharmacokinetic and pharmacodynamics of drugs. With the development and huge successes of HGP project, one of its major applications is emerge of a new research area of pharmacogenomics, which is used in standardization and individualization of drug therapy. In order to fulfill its goal, precision medicine is the key to solve the problem.

Keywords Pharmacogenomics · Precision medicine · Polymorphism · Biomarkers

1.1 Introduction

The individual and ethnic differences of drug effects are very important issues in clinical drug therapy. Besides seemingly obvious factors such as body weight, height, gender, age, drug quality, organ function, disease progress complication, and food/drug interaction, genetics also plays an important role in such area. The difference of drug effects caused by genetic variation mainly comes from polymorphism of coding genes of drug metabolizing enzymes, transporters and drug targets, which will influence their pharmacokinetics and pharmacodynamics. Therefore, they consist of main research field of pharmacogenomics. Furthermore, Precision Medicine Initiative draws more attention of study and application of pharmacogenomics. This chapter will introduce principles of genetic basis, pharmacogenomics, and precision medicine.

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_1

1.1.1 Genetic Concepts of Pharmacogenomics

1.1.1.1 Gene

Gene is the basic element of heritage information. It usually indicates a specific product (e.g., protein or RNA molecule) of function located in a single nucleotide sequence.

Human genes usually consist of two major regions. One is called coding region (5% of genome), including exons and introns. Another is flanking sequence, located in upstream or downstream of coding region, which has regulating effect, consisting of promoter, enhancer, and terminator (Fig. 1.1).

There are 23 pairs of chromosomes in human genome, including 22 pairs of autosomal and one pairs of sex chromosomes. In 1953, British scientists, James D. Watson and Francis H.C. Crick, proposed an earliest model of structure of deoxyribonucleic acid (DNA) according to X-ray diffraction map and related materials. It was published in Nature magazine and draws enormous attention from the world [1]. At the present time, human genome contains about 300 millions of

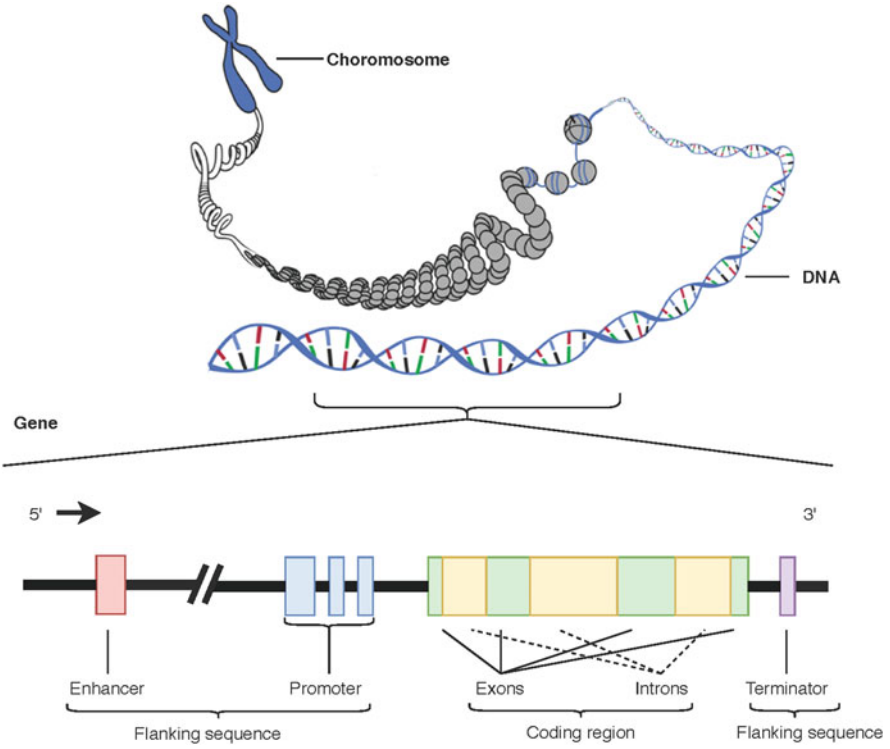

Fig. 1.1 Structure of a typical human gene. (Modified from the original file at https://commons.wikimedia.org/wiki/File:0321_DNA_Macrostructure.jpg)

nucleotides and 27,000 genes. They control individual development, growth, reproduction, and metabolism and play an important role in disease progress.

Gene has three basic characters. First one is self-replication of gene, which is existed as codon in DNA in order to ensure continuity and stability of genetic information in cell division; second one is gene expression, which converts genetic information stored in DNA sequences via transcription and translation to RNA and protein molecules with biological function; third one is gene mutation, which is small change in single nucleotide sequence and number to pave the way of biological evolution and genetic polymorphism.

1.1.1.2 Genetic Polymorphism

Genetic polymorphism means that there are two or more discontinuous mutations and genotypes at the same time and often in a biological population. Genetic polymorphism is very common especially in human, whose construction, expression, and function of gene have been extensively studied.

According to gene variation in human, genetic polymorphism is usually consisted of three categories [2]. (1) DNA fragment length polymorphism (FLP), which is deletion, repetition, or insertion of a single base and results in changes in restriction endonuclease loci and DNA fragment length. It is also called as restriction fragment length polymorphism (RFLP). (2) The polymorphism of DNA repeat sequence (RSP), in particularly short tandem repeats, is small satellite DNA and microsatellite DNA. RSP is mainly manifested in the variation of the number of copies of repetitive sequences. (3) Single nucleotide polymorphism (SNP) is scattered difference of single nucleotide, including the deletion and insertion of a single base, but more frequently the substitution of a single base, which often appears in CG sequence. SNP is a well-studied polymorphism at the present time.

Human genome has about three billion base pairs, in which there are approximately 27,000 genes which can be passed from parent to offspring. Small differences in gene can result in big variation in phenotypes, such as body height, skin color, fingerprint, blood type, and even personality. Variation in specific gene will influence human susceptibility to disease and reaction to drugs (such as pharmacokinetics, pharmacodynamics, and adverse reaction) [3]. Occurrence of polymorphism is a result of multi gene alleles. It is usually considered as polymorphism if frequency of gene mutation is more than 1%, otherwise as natural mutation.

Study of genetic polymorphism will pave the way for clinical medicine, genetics, preventive medicine, and clinical pharmacy. For example, functional impairment or complete loss of drug metabolizing enzymes and transporters by SNP will result in disposition changes of related drugs in vivo. There are variations of number, construction, and function of receptors in certain percentage of individuals, which could affect target protein affinity and finally pharmacological activity of drugs. Some protein and related gene, which determine drug activity, will also influence pathophysiology of diseases. Therefore, personalized use of medication can be met if pharmacotherapy is based on genetic polymorphism. Take hypertension drug

therapy as an example, individual drug selection and dosage adjustment will base on study of genetic polymorphism, in spite of unselective use of ACEI, calcium blocker, or sympathetic receptor blocker.

1.1.1.3 Development of Pharmacogenomics

As early as the beginning of the twentieth century, British scholar Garrod suggested that gene impairment could cause loss of specific enzyme, resulting in the so-called congenital metabolic deficiency. He indicated that personal difference of drug reaction comes from genetic variations [2]. Thereafter, in the 1950s, pharmacogenetics has been raised to study the effect of genetic polymorphism on the individual differences of drug activity. During its development, there were several landmark works as follows: (1) In 1956, Carson found that some patients with genetic G6PD dysfunction in red cell would suffer from hemolytic reaction to Primaquine at therapeutic dosage due to deficiency of reduced glutathione deficiency in red cell [3]. (2) In 1960, Evans developed a phenotype method of isoniazid acetylation by calculating a ratio of acetyl isoniazid to its parent drug, which actually was a classical study in pharmacogenetics [4]. (3) In the late 1970s, the interindividual variability in the capacity to hydroxylate an antihypertensive drug named debrisoquine was reported. It was shown that the deficiency in this metabolism was inherited as an autosomal recessive trait [5]. Later it was demonstrated that both drugs are metabolized by the same enzyme, debrisoquine–sparteine hydroxylase. Since then many substrates and reactions were reported for CYP2D6. When the human *CYP2D6* gene was finally cloned in the late 1980s, it was shown that most of the individuals with deficient capacity to metabolize these drugs carry inactivating mutations in this gene [6, 7].

After the 1980s, development of molecular biology has provided efficient research tools for pharmacogenetics. For example, genes encoding debrisoquin hydroxylation enzyme had been cloned, successfully expressed by vectors and its genetic polymorphism studied (Fig. 1.2) [8]. Thereafter, molecular mechanism of more and more drug metabolizing enzymes, transporters, and receptors has been elucidated one after another. With the development of researches, pharmacological effects of drugs are not only decided by single gene, whereas a consolidated result of multi genes in regulating interaction of drug metabolism, distribution, and efficacy.

In the 1990s, a new era of pharmacogenomics comes from the emergence and rise of genome-wide technology. International human genome project (HGP) was begun on early 1990s and human genome sequencing was completed in 2003, which pave the solid way for the development of pharmacogenomics. The term, pharmacogenomics, appeared in many scientific papers and monographs.

There are some similarity and difference between pharmacogenetics and pharmacogenomics. Pharmacogenetics is primarily focused on effects of genetic polymorphism on drug disposition and efficacy. On the other hand, pharmacogenomics not only deal with single gene and drug effect as in pharmacogenetics,

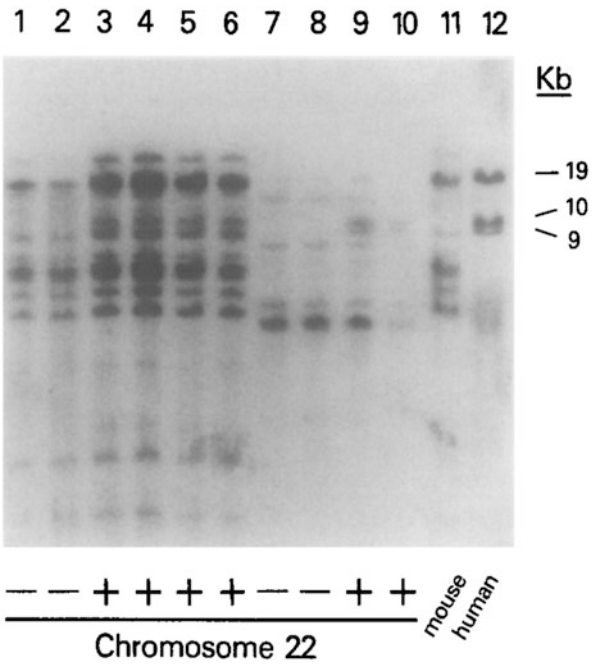

Fig. 1.2 Southern hybridization of the ^{32}P-labeled cDNA probe with EcoRI-digested, size-fractionated DNA isolated from human-rodent somatic cell hybrids and parental cells. Genes encoding debrisoquin hydroxylation enzyme (db1) was localized to human chromosome 22. Human × mouse (lanes 1–6) and human × hamster (lanes 7–10) hybrid cell DNA samples are shown. Parental mouse and human placental DNA samples are presented in lanes 11 and 12 (permission from [8])

but also whole genome and drug development. In this monograph, these two terms are used equally and interchangeably.

1.1.2 Precision Medicine

1.1.2.1 Ethnic and Individual Differences in Drug Therapy

Ethnic factors include genetic and environmental aspects. Different ethnicity has different genetic background, such as different genotype or diverse gene frequency of same genotype. They live in various geographical environments with different culture, food, and habit for a long period of time, which will result in ethnic differences in drug metabolism and effect. Take ethanol metabolism as an example, acetaldehyde level in Chinese is significantly higher than that in Caucasians after same amount of alcohol intake, resulting in higher occurrences of flush face and palpitation. This is because that activity of ALDH2 enzyme, which is responsible for the metabolism of acetaldehyde into acetate, is significantly higher in Caucasian than that in Chinese. Cardiovascular reaction of propranolol in Chinese is more sensitive than that in Caucasians, whereas it is the least in black population. Ethnic differences of drug metabolism and effect depend on therapeutic windows of drugs.

Ethnic difference of drug effect has become one of the important factors influencing drug uses, medication management, clinical trial, and development of new drugs.

Gefitinib, a selective epidermal growth factor receptor (EGFR) inhibitor in the treatment of Non-Small Cell Lung Cancer (NSCLC), was not as effective as expected in Phase III clinical trial in the USA. However, it was found that it is effective in subgroup of Asian population, such as Japanese and Chinese. Further study revealed that NSCLC patient carrying EGFR mutant is more effective than one without mutant (>90% vs <10%). More importantly, frequency of EGFR mutation in Asian NSCLC patient is also significantly higher than in that of Caucasians (30–40% vs <10%).

As compared with ethnic difference of drug effect, individual difference of drug metabolism and pharmacodynamics inside an ethnicity is also significant and important. Take the same example of propranolol, there is onefold difference of its average plasma level between Chinese and Caucasian. However, there is up to tenfold difference of propranolol plasma level either in Chinese or in Caucasian at the same dosage.

1.1.2.2 Human Genome Project

Human genome project (HGP) is a scientific exploring project of large scale, interdisciplinary and multi-countries. It aimed to determine nucleotide sequence of human chromosome which contains 3 billion bases, then map the human genome and identify the genes and their sequences in order to achieve the ultimate goal of deciphering human genetic information [9].

The strategies, ideas, and technologies during HGP development constitute a new area of life sciences—genomics, which is used to study microorganisms, plants, and other animals. HGP is also one of the three major science programs. Other two are Manhattan Atomic Bomb Program and Apollo Moon Landing Program. HGP is the great program in human science history and also called "Moon Landing Program" in life science.

HGP was first proposed by American scientists in 1985, and formally initiated in 1990. Scientists from the USA, British, France, Germany, Japan, and China jointly participated in this project which cost 3 billion US dollars. According to its plan, it would decode all of the 25 thousand human genes and draw the map of human genes. As of April 14, 2013, gene sequencing of 3 billion base had been finished, which was the milestones of successes in HGP project [10].

1.1.2.3 Development of Precision Medicine

With the development and huge successes of HGP project, one of its major applications is to apply their theory and technique to solve the dilemma of ethnic and individual differences in drug therapy. Precision medicine is the key to solve the problem. It was firstly proposed by former American President Obama in his State of the Union Address on January 30, 2015. According to "Precision Medicine Initiative," it would plan to sequence genes of one million of American volunteers and

elucidate the mechanism of disease in order to pave the way of relevant drug development and precision medicine. As he mentioned that cancer pharmacotherapy based on genotype is just as easy as blood transfusion matches blood group and prescribe right drug in right patient at right time just like body temperature measurement [11].

The term of precision medicine is not actually new. It is very similar to individualized or personalized medicine proposed before. The common core of them is to develop individual drug and personalized therapy for specific disease subtype. As NIH director Dr. Collins said that "path to personalized medicine is to accelerate the research and development of biomedicine and provide the latest tools, knowledge and treatment options while doctors treat right patients with right therapy" [12].

Pharmacogenomics provides basic theory and practical tools for precision medicine. The US Food and Drug Administration (FDA) released "Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendation for Labeling" in 2013 [13]. It aims at providing help for pharmaceutical industries in evaluation of effects of human genetic polymorphism on pharmacokinetics, pharmacodynamics, efficacy, and safety during drug development. It also emphasizes the importance and necessity of relevant genetic testing. In FDA website, there is more than 200 information of relationship of genetic biomarkers with drug effects and safety [https://www.fda.gov/drugs/science-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling]. The information has been included in drug labeling. Notably, a guideline proposed by Clinical Pharmacogenetics Implementation Consortium (CPIC) provides the basis for translation of laboratory results of genetic testing into clinical practice. This guideline could be found in a website of "Pharmacogenetics and Pharmacogenomic Knowledge Base" [https://www.pharmgkb.org].

Applied population of precision medicine related genetic testing include: (1) Patients of long-term medication use, such as cardiovascular disease, psychiatric diseases, tuberculosis, immunosuppressive users, etc.; (2) Patients with adverse drug reaction or their family member with severe adverse drug reaction; (3) Special population such as elderly and children; (4) Patients with polypharmacy; (5) The effect of using a certain drug is not ideal and the condition is not well controlled. Therefore, health-care providers should consider genetic and non-genetic factors (e.g., environmental, disease progress, drug interaction, food and organ function) in rationally choosing gene testing in clinical practice.

1.2 Genetic Biomarkers of Pharmacogenomics

The interindividual differences of drug reactions caused by genetic variations mainly come from polymorphisms of genes encoding metabolic enzymes, transporters, and drug targets that affect the in vivo pharmacokinetics and pharmacodynamics of drugs. Therefore, genetic polymorphisms of these genes are focus of pharmacogenomic research and will be briefly reviewed in this chapter.

1.2.1 Drug Metabolizing Enzymes

Drug metabolism is a chemical process, where enzymes play a crucial role in the conversion of one chemical species to another. Structures and concentration variations of enzymes, which may lead to individual differences in drug metabolism, could be determined by genetic factors. Most of metabolic enzymes have clinically significant genetic variations, but only some important and pharmacogenomics-related enzymes will be covered in this chapter.

1.2.1.1 Phase I Drug Metabolizing Enzymes

Cytochrome P450 enzymes (CYP) are one of the main enzyme families that mediate oxidative metabolism of drugs. Several CYP isozymes, e.g., CYP3A4/5, CYP2D6, CYP2C9, and CYP2C19, with highly variable polymorphisms, become the focus of pharmacogenomics research.

CYP3A4/5

CYP3A4 is the most abundant human hepatic and intestinal CYP enzyme and involves in the metabolism of the most drugs. *∗1B* (*-382A>G*) mutation in the promoter region may upregulate the expression of CYP3A4, which has been reported to reduce intestinal absorption of indinavir and increase the clearance of docetaxel. Frequency of *∗1B* is higher in blacks (35–67%) compared to Caucasians (2–10%) and Asians (0%). Asians have other two unique genotypes, *∗18B* and *∗1G*, with frequencies of about 10% and 30%, respectively. *∗18B* causes the change of 293th protein from leucine to proline and then the enhancement of enzyme activity, while *∗1G* do the opposite. Although genetic variations could cause large interindividual differences in CYP3A4 enzyme activity, no clinically significant mutation has been identified after massive studies yet.

Content of CYP3A5 is much lower than that of CYP3A4, but its genetic variations (e.g., *∗3*) have more significant impact on drug metabolism, especially immunosuppressants among Chinese population. Since substrates of CYP3A4 and CYP3A5 are almost identical, increasing the difficulty to distinguish them by either in vitro or in vivo phenotypes, combined *CYP3A5∗3* and *CYP3A4∗1G* genotypes were found to affect pharmacokinetic profile of tacrolimus such as dose-adjusted trough concentrations (C_0/D) among Chinese renal recipients. The pharmacodynamic indicator, incidence of acute rejection during the first year after renal transplantation operation, was also strongly associated with *CYP3A5∗3* [14].

CYP2D6

CYP2D6, although consisting of only 2–4% of liver total CYP content, participates in the metabolism of 25–30% of clinical drugs, such as fluoxetine, nortriptyline, haloperidol, tamoxifen, carvedilol, metoprolol, and codeine. It is also the CYP isozyme which has been studied the earliest and deepest in the field of pharmacogenomics. More than 50 drugs have been requested by FDA to pay attention to the impact of genetic polymorphisms on drug dosage. Since CYP2D6 is uninducible, genotype is the key factor determining its activity. Around 80 mutations have been identified so far. Among which, those causing loss of enzyme activity are called null alleles. *∗4* is the most common null one among Caucasians with an approximately frequency of 18%, and 3–6% in blacks and 0.5% in Asians, respectively. Some other mutations, decreasing the activity, are called impaired function alleles, such as *∗10* allele, which has a high frequency in east Asians (45%). Increased metabolic capacity, caused by *CYP2D6* gene multi-copy, is particularly common in blacks, with a frequency of up to 10%. CYP2D6 metabolism phenotypes, determined by above mutations, can be divided as follows: poor metabolizer (PM) carrying null alleles, intermediate metabolizer (IM) with impaired function alleles, extensive metabolizer (EM) as wild type, and ultrarapid metabolizer (UM) caused by multiple gene copy. A large amount of research has demonstrated the importance of *CYP2D6* phenotypes in clinic. Cai et al. studied the stereoselective metabolism of propafenone (PPF) in Chinese population after oral administration [15–17]. Two times higher C_{max} and AUC and 50% lower clearance (Cl) of both enantiomers among IM phenotype were observed when comparing to EM and UM phenotype. IM phenotype is identified among Chinese population with a frequency of as high as 36%, while PM phenotype is rare (1%), which is opposite to Caucasians. The result indicated the contribution of IM phenotype to the interindividual difference and the intolerability of certain CYP2D6 substrates among Chinese population.

CYP2C9

CYP2C9, an important hepatic metabolic enzyme, accounting for 18–30% of liver total CYP content, has at least 34 known mutant alleles, of which 7 loci have significantly ethnic difference. *∗2* allele, a point mutation in exon 3, could change the 144th arginine to cysteine, and *∗3* allele in exon 7 could result in the 359th isoleucine to leucine. Both of them are more common in Caucasians, with frequencies of 13% and 7%, respectively, and only 3% and 2% in blacks. *CYP2C9∗2* is rarely found in Asian populations, while *∗3* is found in about 4% of them only as heterozygous. In vitro studies showed that enzyme activities of the two alleles are only 12% and 5% compared to that of wild type, respectively. Drugs affected by *CYP2C9* genotypes include warfarin, phenytoin, flurbiprofen, and celecoxib. The influence on the warfarin was first described in the late 1990s, confirmed by massive follow-up researches, including genome-wide association studies (GWAS). Mutants *∗2* and *∗3* account for around 10% of the variation in warfarin dose requirement, but

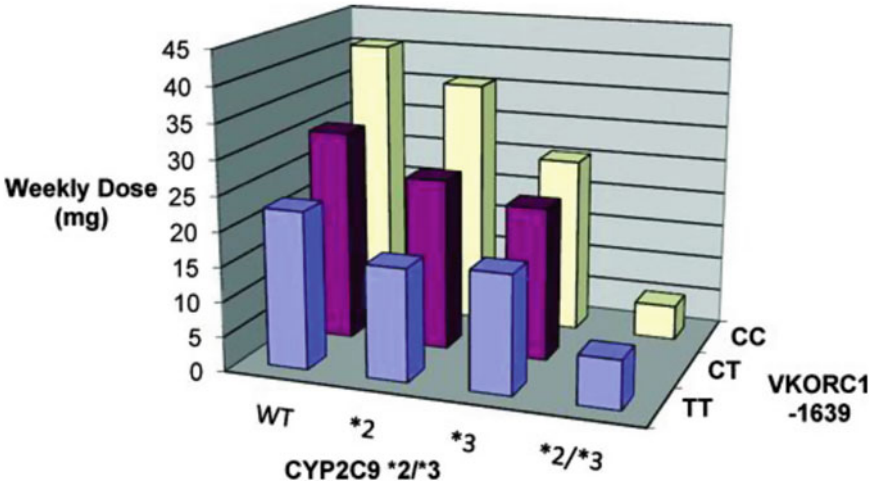

Fig. 1.3 Weekly stable dose for different CYP2C9 and VKORC1 genotypes. A total of 216 subjects were recruited in this study. *WT* wild type (permission from [28])

about 35% of the dose variability could be explained by variants of *CYP2C9* and *VKORC1* together (Fig. 1.3, See Sect. 1.2.3.2), and when they are combined with clinical data, up to 50% can be explained [18, 19] (Fig. 1.3).

CYP2C19

CYP2C19 involves in the metabolism of 5% of drugs. So far, seven null alleles (∗*2*–∗*8*) have been reported. 93% of Caucasians and 75% of Asians with *CYP2C19* defect carry ∗*2* allele, a *681>A* mutation in exon 6, resulting in the generation of premature termination codon and then translation of incomplete enzyme protein. The other 25% of Asians with *CYP2C19* defect have ∗*3* allele in exon 4, which could also lead to a premature termination codon. Overall, PM phenotype caused by *CYP2C19* non-function mutations has a high frequency in Asians (15%), while it is only 2–5% in Caucasians. Conversely, ∗*17* (*-806C>T*) mutation could significantly increase the transcriptional activity of CYP2C19 and act as UM phenotype. This allele is more common in Caucasians (21%) and Africans (16%), compared to east Asians (2.7%). Clinical evidence indicates that interindividual difference in CYP2C19 enzyme activity mainly depends on genetic polymorphisms, and further affects many drugs, including clopidogrel, prasugrel, citalopram, voriconazole, and proton pump inhibitors (omeprazole and lansoprazole). CYP2C19 mediates the transformation of inactive clopidogrel to its active metabolite, of which genetic variants are significantly associated with its pharmacokinetics and pharmacodynamics. Patients receiving clopidogrel and carrying ∗*2* or ∗*3* loss-of-function alleles had a higher rate of subsequent cardiovascular events [20]. This impact should be particularly noted among Chinese patients regarding the high incidence of the two alleles [21].

1.2.1.2 Phase II Drug Metabolizing Enzymes

Phase II drug metabolizing enzymes, mainly transferases, play an important role in biotransformation of endogenous compounds and xenobiotics to more easily excretable forms. Major phase II enzymes include uridine diphosphate glucuronosyltransferase (UGT), thiopurine methyltransferase (TMPT), and N-acetyltransferase (NAT).

Uridine diphosphate glucuronosyltransferase (UGT)

UGTs are the most important human phase II metabolic enzymes. Among which, genetic polymorphisms of UGT1A1 have been studied extensively, especially *∗28* allele, occurring in the TA cassette of promoter region, which increases TAs from 6 to 7, then significantly reduces the rate of transcription and finally decreases expression of enzyme. Frequencies of *UGT1A1∗28* homozygotes are 9% among American Caucasians and 23% among African Americans, while only 2% in Chinese. Impact of *UGT1A1∗28* genotype on the side effects of irinotecan was the first pharmacogenomic information added to drug package by FDA [22]. In addition to drugs, UGT1A1 also metabolizes many endogenous substances, such as bilirubin. Genetic defect of *UGT1A1* leads to impaired glucose hydroformylation of unconjugated bilirubin, resulting in hyperbilirubinemia, also known as Gilbert's syndrome. Among *∗28* homozygous patients compared to heterozygous and wild type patients, nilotinib, for the treatment of chronic myeloid leukemia disease (CML), and a potent inhibitor of UGT1A1, was found to cause 4.5–18 times of elevated bilirubin risk above CTCAE grade 3 after treatment, suggesting nilotinib dose decline among *UGT1A1* deficiency patients [23].

Thiopurine methyltransferase (TMPT)

Of the 21 polymorphisms that affect TMPT activity, 18 are non-synonymous SNPs, and three of which, *∗2*, *∗3A* and *∗3C*, could explain approximately 80–95% of the cause of medium to low activity of TMPT enzyme. *∗3A* is the most common genotype in Caucasians (about 5%) and consists of two non-synonymous SNPs, *Ala154Thr* and *Tyr240Cys*. The structural change in enzyme protein leads to accelerated protein degradation. But these genotypes are rare in Chinese population. The most common genotype among east Asians is *∗3C* (2%), a SNP in the 240th codon. Homozygous or combined heterozygous carriers of *∗2, ∗3A,* and *∗3C* have low or none enzymatic activity, while heterozygous carriers with single mutation are moderately active. TMPT is responsible for the detoxification process of 6-mercaptopurine, azathioprine, and 6-thioguanine, and these defective genotypes may increase the likelihood of toxicity after thiopurines treatment, such as fatal myelosuppression. Thus, patients carrying defective genotypes should reduce dose

to avoid toxicity. This case is known as a representative example revealing the significance of pharmacogenetics.

N-acetyltransferase (NAT)

NAT has two isozymes: NAT1 and NAT2, of which NAT2 is mainly expressed in liver and is responsible for the phase II conjugate metabolism of drugs including isoniazid, hydralazine, and sulfamethazine. The wild haplotype of *NAT2*, *∗4*, acts as rapid acetylation, while poor acetylation is determined by haplotypes of *∗5B, ∗6,* and *∗7*. Frequency of *∗4* in Caucasians is lower (20–25%) compared to African Americans, Chinese, and Japanese (36–41%, 50% and 70%, respectively). *∗5B* is more common in Caucasians (44%), followed by blacks (25–27%) and Asians (2–6%). Frequency of *∗6* is similar in all races (18–31%). *∗7* is more frequent among Asians (10–19%) while other races are rare. In the early 1950s, soon after the application of isoniazid for treatment of tuberculosis, its large interindividual differences was found in acetylation metabolism, and patients receiving isoniazid could be divided into rapid or poor metabolizing group according to the ability of acetylation. Further studies revealed that this ability is largely determined by the NAT haplotypes, marking this genetic influence on drug acetylation the first discovered pharmacogenetic phenomenon.

1.2.2 Drug Transporters

As an essential part of in vivo process, transmembrane transport of drugs mainly relies on passive diffusion, but more studies have found that transporter-mediated transport is also important, sometimes even decisive. Drug transporters are divided into two super families: ATP-binding cassette (ABC) and solute carrier class (SLC). ABC relies on adenosine triphosphate (ATP) to provide energy to transport molecules against concentration gradient, while SLC depends on the cell membrane potential difference or ion concentration difference. In addition, transporters can also be divided into two categories according to the direction of transportation: efflux transporters transporting their substrates from intracellular to extracellular side and uptake transporters from extracellular to intracellular side. ABC transporters are all efflux transporters, and SLC superfamily, apart from multidrug and toxic compound extrusion protein (MATE), are all uptake transporters.

1.2.2.1 Efflux Transporters

P-gp (MDR1) and breast cancer resistant protein (BCRP), members of the ATP-binding cassette super family, responsible for effluxing parent drug and

metabolites out of cell, involve in drug resistance by diminishing the desired therapeutic or biologic effect.

P-glycoprotein (P-gp)

P-gp, also known as MDR1, is the first transporter that has been thoroughly studied. A lot of variations have been found in its encoding gene *ABCB1*, but only a few could affect P-gp function. Non-synonymous mutation *2677G>T* (*p.893A>S*) is found to enhance P-gp transport activity due to the protein structural change. Another important SNP, *3435C>T* in exon 26, could reduce the expression of P-gp in vitro. Clinical data similarly found that *3435T* allele could decline intestinal P-gp efflux capacity, resulting in decreased intestinal absorption and increased plasma concentration of digoxin, a typical substrate of P-gp. Another important substrate of P-gp, cyclosporin A, could also be affected [23]. But the above impacts of genetic variants could not be confirmed in all clinical researches, suggesting that genetic variants of P-gp are not decisive factors affecting the pharmacokinetics and pharmacodynamics of drugs, but often related to other internal and external factors. In addition, haplotypes should also be considered regarding the effects of genetic variations on P-gp function, especially the haplotype composed of *1236-2677-3435* alleles, could result in different response in chronic myeloid leukemia patients treated with imatinib [24]. The gene frequency has significant racial differences. The two main haplotypes among Caucasians are *TTT* and *CGC*, while African Americans are basically *CGC* type, and *CAC, CGC,* and *TTT* are more common among Japanese.

Breast Cancer Resistant Protein (BCRP)

BCRP, encoded by the *ABCG2* gene, is distributed across different organs, such as intestine, liver, kidney, blood–brain barrier and placenta. Some *ABCG2* mutations weaken transport function of BCRP, such as *c.34G>A, c.421C>A, c.1465T>C* and *c.1291T>C*, etc., wherein *c.421C>A* occurs relatively frequently among Asians and Caucasians (8–35%) and has more clinical studies. Steady state plasma concentration of gefitinib among *c.421A* mutant patients is higher than that of wild type patients, due to mutation mediated impaired efflux function of BCRP located on apical membrane of intestinal epithelial cells, which leads to increased absorption of gefitinib [25].

1.2.2.2 Uptake Transporters

Uptake transporters function in intestinal and hepatic absorption, blood–brain barrier penetration and excretion into the bile and urine, of which functional alteration may lead to declined blood concentrations of the medication and cause risk for therapeutic failure.

Organic Cation Transporter (OCT)

Important members of the OCT family include OCT1 and OCT2, wherein OCT1 is highly expressed on the basal membrane of hepatocyte and plays a pivotal role in the hepatic uptake of the type 2 diabetes drug metformin. Some non-synonymous variations on the encoding gene *SLC22A1* can reduce the transport of metformin, such as *c.1256delATG* (*p.420del*) and *c.181C>T* (*p.R61C*). Plasma concentration of metformin among mutant individuals is higher than that of wild type individuals, indicating less hepatic uptake and lower efficacy of metformin in these patients, which could partly explain the large interindividual differences in the hypoglycemic effect of metformin. OCT2, predominantly expressed on the basal membrane of epithelial cells of kidney proximal tubule, is responsible for uptake of weakly basic substance. Substance could further be transported to tubular lumen by passive diffusion or by efflux transporters, making the uptake of OCT2 the first and possibly the rate limiting step in the active secretion of some drugs. Many variations are found on the OCT2 encoding gene *SLC22A2*, but frequencies of most of them are very low. *c.808G>T* (*p.270A>S*) is the only variant with frequency of higher than 10% among various races. Protein structural change induced by it could reduce the transport activity of OCT2 in vitro. Clinical studies found similar results. In Chinese patients with type 2 diabetes undergoing metformin therapy, *c.808G>T* variant increased plasma lactate levels (a biomarker for metformin treatment) and the incidence of hyperlacticaemia [26].

Organic Anion Transporting Polypeptide (OATP)

OATP is also known as SLCO (solute carrier organic transporter family). OATP1B1 and OATP1B3 of the OATP1B subfamily have been extensively studied due to their pivotal role in drug disposition. OATP1B1 is mainly expressed on the basal membrane of hepatocytes and is responsible for the uptake of endogenous and exogenous substrates from the portal vein into liver. *SLCO1B1*, the encoding gene of OATP1B1, is discovered to have some SNPs affecting OATP1B1 transport function. *c.388A> G* and *c.521T>C*, the two most common SNPs, constitute four haplotypes of *SLCO1B1*: *∗1a (c.388A-c.521T), ∗1b (c.388G-c.521T), ∗5 (c.388A-c.521C),* and *∗15 (c.388G-c.521C)*. In vitro studies suggested that haplotypes of *∗5* and *∗15* carrying *c.521C* mutant allele decrease OATP1B1 transport activity and reduce hepatic uptake of substrate, resulting of increased drug exposure in systemic circulation. Statins, of which the target organ is liver, are most affected by *SLCO1B1* genotypes, since their efficacy is determined by liver concentration, while adverse reactions (such as myopathy) are associated with systemic exposure. Impaired function of OATP1B1 could decrease efficacy and increase risk of adverse reactions of statins [27].

Overall, pharmacogenomics study of drug transporters is not as mature and in-depth as that of metabolic enzymes. But further research and application are still necessary regarding the significant guidance to clinical practice in the future.

1.2.3 Drug Targets

The most drug targets are proteins, including receptors, enzymes, transporters, and proteins involved in cellular biological processes such as signaling and cell cycle regulation. Compared to reported genes involved in drug pharmacokinetic process, pharmacogenomic research about drug target is rather rare. Although targets of drugs are some specific receptors or enzymes, their efficacy is often related to several different proteins on a complicated path, and any link in the path could have genetic variations affecting efficacy. However, current studies mainly focus on pivotal drug targets, and influence of genetic variation of the entire path could not be revealed.

1.2.3.1 Receptors

Current pharmacogenomic research mainly focuses on G-protein coupled receptor. Other receptors, such as ligand-gated ion channels and receptor tyrosine kinases, are rarely studied.

Dopamine Receptors

Dopamine receptor is the main target of typical antipsychotics. There are five subtypes of dopamine receptors, from D1 to D5, of which D2 and D3 are most studied. D2 receptor is the primary target for first-generation antipsychotics such as chlorpromazine and haloperidol. Its encoding gene is *DRD2*, and antipsychotic effects are associated with its two SNPs in the coding region, *Ser311Cys* and *-141-Cins/del*, which could lead to decreased receptor function or receptor protein expression, respectively, and then reduced response of D2 receptor to psychotropic drugs. *DRD2* gene polymorphisms are also associated with treatment-induced tardive dyskinesia. *-141-C* deletion genotype could induce higher risk of developing tardive dyskinesia, probably because of relatively high receptor occupancy of drugs regarding low receptor expression level. *DRD3* is the encoding gene of D3 receptor. *Ser9Gly* variant could enhance the binding between D3 receptor and dopamine. Receptors of *Gly* mutant type have been clinically confirmed faster binding with drugs, leading to more pronounced efficacy and side effects.

Adrenergic Receptors

Adrenergic receptors play pivotal roles in cardiovascular and respiratory systems in the regulation of many important physiological processes, and thus is an important drug target, particularly β receptors. Specific antagonists and agonists have been used for the treatment of different diseases. β1 receptor is the major adrenergic receptor type on heart, and the encoding gene is *ADRB1*. *Gly398Arg*, a common

non-synonymous SNP in the 398th codon, changes glycine to arginine. Clinical studies indicate that patients carrying *Gly398* homozygous have worse reaction to β blockers, requiring dose increment to achieve therapeutic effect. β2 receptor, an important target for the treatment of asthma, is encoded by highly polymorphic gene *ADRB2*. *Gly16Arg* mutation in the 16th codon, resulting in the translation of glycine (Gly) to arginine (Arg), is relatively common and is the subject of most clinical studies. Cells studies and clinical trials have shown that *Arg* genotype could reduce efficacy of short-acting β receptor agonists, while long-acting β receptor agonists are less affected. In addition, clinical studies have found no significant influence of *ADRB2* genotype on β blockers.

1.2.3.2 Enzymes

The majority of drugs act on enzymes as inhibitors and most of these are competitive. Genetic polymorphisms of these enzymes have been demonstrated to have important implications on drug efficacy as well.

Vitamin K Epoxide Reductase (VKOR)

VKOR, the most studied drug target enzyme among current pharmacogenomic researches, is the target of coumarins (such as warfarin) and encoded by *VKORC1* gene. Mutation in the coding region of *VKORC1* is rare, and occurs more commonly in noncoding region. *-1639G>A*, an SNP influencing gene expression of *VKORC1*, could lead to individual differences of VKOR enzyme protein content in liver, while VKOR protein level is a direct determinant of the dose required for anticoagulants. The *-1639A* allele has lower transcription level and lower dose requirement than *G* allele. A large number of independent clinical studies have proven effective dose of warfarin is closely related to *-1639G>A*. As described in Sect. 1.2.1.1, it is estimated that about 20% of interindividual differences of warfarin dosage can be attributed to this SNP, of which the contribution is more significant than *CYP2C9* variants (Fig. 1.3) [28]. Other coumarin anticoagulants, such as acenocoumarol and phenprocoumon, are similarly affected by *-1639G>A*. *G* allele appears more frequently than *A* in Europeans, while East Asians do the opposite, leading to significantly lower dose requirement of coumarin anticoagulants among East Asians than Europeans.

Angiotensin Converting Enzyme (ACE)

Another well-studied drug target enzyme is ACE, which is an important component of the renin-angiotensin-aldosterone (RAS) system, responsible for the removal of two amino acids at the C-terminus of angiotensin I to produce angiotensin II. Due to the presence or absence of the 287 bp Alu repeated sequence, the 16th intron of ACE

gene has two genotypes, insertion (I) or deletion (D). The deletion (D) genotype could result in higher plasma ACE levels than the insertion (I) genotype. Although early clinical studies have found that enalapril has better antihypertensive effect on *II* genotype than *DD* genotype, or that irbesartan has better antihypertensive effect on patients with *D* allele than *I*, subsequent studies have not confirmed *I/D* polymorphism of ACE gene could affect the antihypertensive effect of ACE inhibitors or angiotensin II blockers.

1.3 Conclusion and Prospect

The inter-ethnic and interindividual differences of drug reactions caused by genetic variations mainly come from polymorphisms of genes encoding metabolic enzymes, transporters, drug targets and signal pathway that affect the in vivo pharmacokinetic and pharmacodynamics of drugs. Pharmacogenomics in precision medicine era is new clinical area of standardization and individualization of drug therapy. It aims to individual and ethnic differences of drug effects. In order to fulfill its goal, precision medicine is the key to solve the problem.

However, during our exploration and practice, we found that there are some problems to be solved urgently. Firstly, how to protect personal information privacy? Precision medicine often collects individual data, such as genomics and metabolomics. It is vital to protect patient's privacy during recording, sharing, and processing of pharmacogenomic data from electronic medical record system. It needs not only consensus of interdisciplinary collaboration of researchers and medical staffs, but also awareness of government, individual, family, enterprise, and research institute. Secondly, construction of big data platform is needed. Professional personnels are required to construct precise data website for the management of pharmacogenomic data, high throughput research data, patient clinical information, and clinical samples. Biological sample bank from different area can be shared through resource integration. Thirdly, standardization of detection technology will be integrated. For example, gene detection of drug metabolizing enzymes and drug effect targets as well as LC-MS analysis of parent drug and its metabolite will meet the requirement of China National Accreditation Service for Conformity Assessment in China. It ensures construction of a laboratory with standardization and quality control. Lastly, high quality staff for detection and analysis is needed. They should often concern the updating of relevant guideline of disease diagnosis and give advice in result report to guide clinical diagnosis and treatment. In order to achieve such goal, a teamwork of clinical medicine, diagnostic medicine, pathology, pharmacy, and genetics is highly warranted.

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Chapter 2
Pharmacogenomics in Cardiovascular Diseases

Xiaoqiang Xiang and Zhiping Jin

Abstract Cardiovascular disease (CVD) is one of the most serious health problems, particularly in developed countries. CVD includes various abnormal conditions such as hypertension, hypercholesterolemia, congestive heart failure, cerebrovascular disease, and coronary heart disease. Many drugs have been developed to combat CVD. However, not all the drug therapy of CVD could have satisfactory results. The large interindividual variability of cardiovascular drugs could be often explained by the genetic variation along human genome. Some important findings of pharmacogenomics have been confirmed in clinical studies of large scale, presenting a big potential in clinical application. This chapter summarizes pharmacogenomics knowledge in some of the most commonly used drugs in the treatment of CVD.

Keywords Cardiovascular disease · Pharmacogenomics · Lipid-lowering drugs · Antihypertension drugs

2.1 Introduction

Cardiovascular disease (CVD) is one of the most serious problems threatening human health all over the world, particularly in the developed countries. The cause of CVD is a complex process involving various genetic and environmental factors. Currently, drugs are an important option to treat CVD in addition to lifestyle change and surgery. Large interindividual variability has been observed for the efficacy of cardiovascular drugs due to the heterogeneous cause of CVD. Thus, it is important to identify specific patients who are most likely to respond to a certain cardiovascular

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_2

drug. In another word, pharmacotherapy of CVD is being moved to an era of precision medicine. Pharmacogenomics appears to be a very powerful tool to achieve this goal. It can help to identify the genetic determinants of drug efficacy and adverse reactions. This chapter will describe how some genetic variants can affect some important cardiovascular drugs.

2.2 Pharmacogenomics of Lipid-Lowering Drugs

Blood lipids are the targets of various drugs since they presents risk factors of different CVDs. Much efforts have been made to find the genetic variants which can help to optimize the clinical use of these lipid-lowering drugs, particularly statin drugs.

2.2.1 Statins

3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, statins, reduce the synthesis of cholesterol in the liver by competitively inhibiting HMG-CoA reductase activity. Placebo-controlled statin trials consistently demonstrate that every 20 mg/dL reduction in cholesterol is associated with a 10–15% reduction of annual incidence rates for vascular events [1]. Statins are one of the most important pharmaceutical intervention for the primary and secondary prevention of cardiovascular diseases (CVD) by increasing uptake of low-density lipoprotein cholesterol (LDL-C) from blood and lowering blood LDL-C concentrations of and other apo-B-containing lipoproteins, including triglyceride (TG)-rich particles. According to a large scale Cochrane review published in 2013 [2], it revealed that the use of statins reduced the all-cause mortality by 14%, CVD events by 27%, fatal and non-fatal coronary events by 27%, and stroke by 22% per 1 mol/L LDL-C reduction. Due to given benefits in reducing cardiovascular risk, acceptable safety profile, and cost effectiveness, statins became one of the most extensively prescribed therapeutic drugs. Scholars once extrapolated that over 1 billion patients worldwide may receive statins therapy [3].

Since lovastatin was first launched in 1987, a number of different statins have been developed. Different statins have different pharmacokinetic properties and varying potencies. Even the same statin, the plasma concentrations can be various among the patients with same dose. It is reported that as much as 45-fold variability in plasma concentration was observed in patients on the same atorvastatin and rosuvastatin daily dose [4]. Current available evidence from meta-analyses suggests that the clinical benefits is largely independent of the type of statin, but depends on the extent of LDL-C lowering. The degree of LDL-C reduction is dose dependent and varies between the different statins. Besides poor compliance, the poor response

to statin therapy may be explained by a genetic background of statin uptake and metabolism in the liver.

Although, statins have overall good safety profile and are generally well tolerated, the adverse effects should be considered when statins are prescribed. The development of musculoskeletal symptoms (such as muscle weakness, myopathy) is the most common adverse effects associated with statin therapy, and it is a barrier to statin treatment as well. Some genetic variants have been linked to the occurrence and severity of adverse events. The choice of drug, starting dose, and titration dose remains challenging.

The major genetic variants associated with statin efficacy and safety will be described in the following part.

2.2.1.1 Genetic Variants that May Affect the Efficacy of Statins

Statins act by reducing cholesterol synthesis in the liver due to inhibition of HMG-CoA-reductase, the rate-limiting enzyme in the cholesterol synthetic pathway. Cholesterol homeostasis is complex and there are a number of candidate genes that may influence the effectiveness of statin therapy. Below are some genes that may cause the variation in response to statins, but not limited.

APOE/C1/C2

Apolipoprotein E (APOE) is a constituent of triglyceride-rich chylomicrons, very low-density lipoprotein particles, intermediate-density lipoproteins, and a subclass of high-density lipoprotein cholesterol (HDL-C). Three major Apolipoprotein E isoforms are coded by three alleles at the APOE gene, designated as e2, e3, and e4 (rs7412 and rs429358 variant). The most common allele is e3, which is found in more than half of the general population [5]. The APOE gene maps to a region of chromosome 19 in a cluster with APOC1 and APOC2. E2 carriers showed a trend for greater reductions in total cholesterol (TC), LDL-C, and triglycerides than the e3 homozygotes and the e4 carriers. Increase in HDL-C levels with statin therapy was greatest in e2 carriers, followed by the e4 carriers and then by e3e3 homozygotes. However, these reductions and increases did not differ significantly among the three genotypes. But in subgroup analysis, patients taking atorvastatin with e4 carriers had statistically significant reductions in LDL-C than e3e3 homozygotes. Among the users of atorvastatin, e4 carriers had statistically siginificnt increases in HDL-C than the other genotypes. For the male gender showed a significantly greater reduction in triglyceride levels for e2 carriers than other genotypes [6].

CYP7A1

CYP7A1, cholesterol 1 alpha-hydroxylase, is the first and rate-limiting enzyme in the classic bile acid synthetic pathway. CYP7A1 gene polymorphism influenced the LDL-lowering significantly in Chinese Han patients taking atorvastatin [7]. The same result that the patients with wild-type genotypes of CYP7A1 (rs3808607) showed significantly greater LDL-cholesterol reductions in response to atorvastatin therapy was also found in the Indian population [8]. As to the clinical utility, further researches are required.

PCSK9

Protein convertase subtilisin/kexin type 9(PCSK9) is an enzyme which mediates the degradation of LDL receptors (LDLRs). Therefore, individuals with PCSK9 loss-of-function (LOF) variants would have better LDL-C response to statins because LDLR degradation is reduced. PCSK9 variant rs11591147 was associated with a 55.6% increase in LDL-C reduction compared with non-carriers ($P = 0.0024$) Africa Americans; the association was also present in European Americans. Another LOF variant, rs28362261 (N425S) was associated with statin response ($P = 0.0064$) and off-treatment LDL-C levels ($P = 0.067$) with borderline significance. Meanwhile, population-specific allele frequency in PCSK9 genetic variants were observed [9]. Rs17111584 variation also has been reported associating with reduced response to statin therapy [10].

KIF6 Gene

KIF6 is a member of the superfamily of kinesins, which are proteins that mediate the intracellular transport of organelles, complex proteins, and mRNAs. The KIF6 variant rs20455 was initially described as an independent risk factor for CVD risk [11]. It was reported that being a carrier of the c.2155T> C variant of the KIF6 gene negatively impacts patient responses to statin treatments. A less pronounced decrease in LDL-cholesterol in the case of simvastatin and atorvastatin and less pronounced increase in HDL cholesterol in the case of rosuvastatin were observed with respect to non-carriers [12].

HMGCR Gene

Statins bind to the catalytic domain of HMG-CoA-reductase (HMGCR), the rate-limiting enzyme of the cholesterol biosynthesis pathway, blocking access of the natural HMG-CoA substrate to the enzyme [13]. There are several variants have been identified that may affect statin efficacy link to HMGCR [14, 15]. One variant, rs3846662, was associated with statin responsiveness in some trials. In another

study, it was reported that rs3846662 polymorphism and the alternative splicing of HMGCR mRNA significantly impacted women's response to statin therapy [16]. Rs1920045 carriers would be predicted to have reduced cholesterol lowering with statin treatment [17].

2.2.1.2 Genetic Variants Relating to Adverse Drug Reactions (ADRs) of Statins

In general, statins are safe and well tolerated, but there are still 25–50% of patients with coronary artery disease noncompliant after 1 year's medication mainly because of ADRs [18]. Musculoskeletal symptoms, such as myopathy, are the most common adverse effects may lead to nonadherence of statin therapy. One observational study, which carried out in 7924 hyperlipidemic patients receiving high-dosage statin therapy, reported that the occurrence of muscular symptoms was 10.5%, with a median time of onset of 1 month following initiation of statin therapy [19]. Though the rate of statin-induced myopathy is low, the number of patients with statin-related muscle symptoms may be substantial due to the high prevalence of cardiovascular diseases and the wide use of statins. On the other hand, when the patients discontinue statin therapy for ADRs, the risk of cardiovascular events will be higher. Hence, understanding the mechanism of statin-induced ADRs has great significance to reduce statin toxicity and optimize patients' adherence. Besides older age, low body mass index, female gender, higher dosage, metabolic comorbidities, intense physical exercise, interactions with other drugs, genetic factors contribute a lot to the risk of developing muscle toxicity during statin therapy [20]. In the following section, some candidate genes influencing the development and severity of statin-associated muscle toxicity will be discussed.

SLOC1B1

The gene most widely investigated is *SLCO1B1* gene. The *SLCO1B1* gene locates on the chromosome 12 (Chr12p12.2) occupying 109 kb, which encodes the organic anion transporting polypeptide 1B1 (OATP1B1). OATP1B1, which has been shown to regulate the hepatic uptake of statins, is predominantly expressed in the liver on the basolateral side of the sinusoidal membrane and has wide substrate specificity. A genome-wide study was carried out in patients taking simvastatin 80 mg daily. 85 subjects with definite (i.e., muscle symptoms, with creatine kinase levels that were more than 10 times the upper limit of the normal range) or incipient (a creatine kinase level that was more than both 3 times the upper limit of the normal range and 5 times the baseline level, plus an alanine aminotransferase level that was more than 1.7 times the baseline value without an elevated alanine aminotransferase level alone at any other visit) myopathy and 90 controls were enrolled, as part of a trial involving 12,000 participants. Meanwhile, replication was tested in a trial of 40 mg of simvastatin daily involving 20,000 participants. The subjects who are

taking high doses of statins (80 mg simvastatin and some other high-dose statin regimens) and who have the C allele of the rs4149056 (521T>C) polymorphism may produce particularly high risks of myopathy. The investigators found five other nonsynonymous variants in *SLCO1B1*. Rs2306283 G allele and rs3471512 C allele were associated with lower risks of myopathy that were of borderline significance ($P = 0.03$ and $P = 0.06$, respectively), whereas rs11045819 did not appear to influence the risk [21]. Atorvastatin, lovastatin, fluvastatin, and simvastatin are lipophilic and pravastatin and rosuvastatin are hydrophilic, which makes the former statins at higher risk of drug interaction.

2.2.2 Ezetimibe

Ezetimibe blocks the Niemann–Pick C1-like protein 1, which is responsible for the intestinal absorption of dietary sterols. Clinical studies indicated that the systemic exposure of ezetimibe was influenced by the *SLCO1B1* polymorphism, whereas no effect of *SLCO1B1* genetic variation was observed on the effect of ezetimibe [22]. Furthermore, *NPC1L1* gene encoding the target of ezetimibe had genetic variations which could affect the LDL-C response of ezetimibe therapy [23]. Generally, a small number of pharmacogenomics studies have been conducted for ezetimibe. Thus, the exact effect of genetic variations on ezetimibe pharmacokinetics and pharmacodynamics warrants further research.

2.3 Pharmacogenomics of Anti-Hypertension Drugs

Although various drugs are available to treat hypertension, about half of patients could have their blood pressure controlled as expected. Novel strategies are still desired to improve antihypertensive treatment in addition to the current guideline in clinical practice. Antihypertension drugs were the hot topics of the early pharmacogenomics research and have attracted much attention. Some significant findings have been applied in clinical practice routinely.

2.3.1 β-Blockers

Beta-adrenergic antagonists (β-blockers) are commonly used to treat patients with various cardiovascular diseases such as hypertension, angina pectoris, MI, and cardiac arrhythmias. Several genes along the pharmacokinetic and pharmacodynamics pathway have been found to influence the response of β-blockers, such as CYP2D6, beta-1-adrenergic receptor (*ADBR1*), the beta-2-adrenergic receptor (*ADBR2*), and *GRK5*. The most significant and earliest example indicating racial differences in drug response is a β-blocker, namely, propranolol. Dr. Zhou

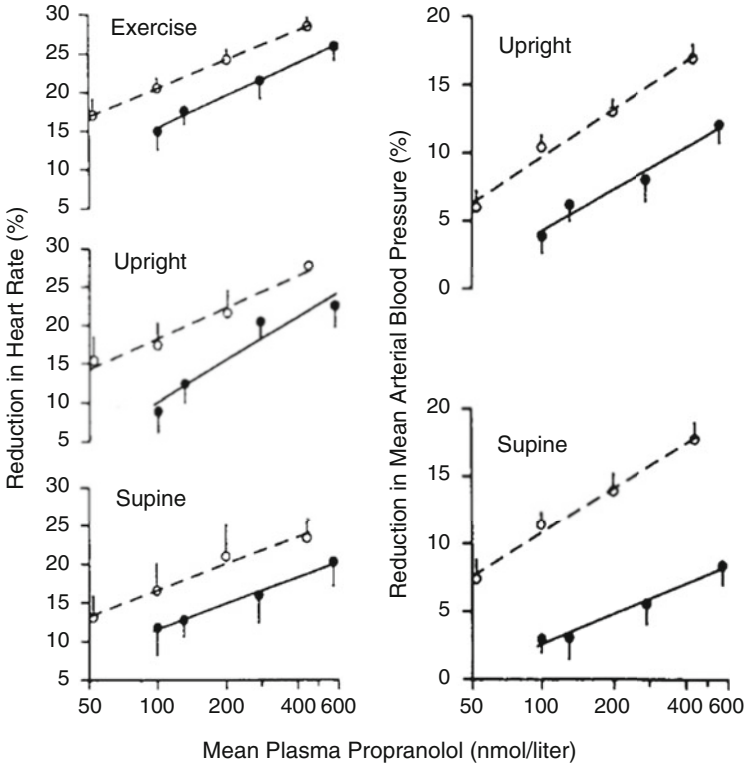

Fig. 2.1 Reduction in heart rate and blood pressure in relation to propranolol concentration, body position, and exercise testing. Open circles represent the means (±SEM) for the group of 10 Chinese subjects, and solid circles the means for the group of 10 white subjects. "Exercise" values were recorded at the completion of treadmill testing. The scale for the drug concentrations is logarithmic

Hong-hao's clinical study found that the subjects of Chinese descent had greater sensitivity to the effects of propranolol by more than twofold as indicated by the reduction of heart rate as shown in Fig. 2.1. Consistently, the Chinese subjects had higher plasma exposure and lower clearance of propranolol compared to the American white men [24].

2.3.1.1 CYP2D6

The metabolizing enzyme of CYP2D6 is involved in the metabolism of several β-blockers, including metoprolol, carvedilol, and propranol. Thus, the genetic variation of *CYP2D6* has been observed to affect the pharmacokinetics of these β-Blockers [25–27]. Generally, CYP2D6 dysfunctional alleles defining the poor metabolizers (PM) could elevate the exposure of metoprolol by four to six-fold and prolong the half-life by two to three-fold [28]. Therefore, a 70–75% reduction of

normal dose was recommended for metoprolol by Dutch Pharmacogenetics Working Group Guidelines, while the metoprolol dose for ultra-metabolizers (UM) was proposed to be increased to about 2.5 times of the normal dose [29]. The CYP2D6 PMs had significantly greater reduction of heart rate, diastolic blood pressure, and mean arterial pressure as well as 4.9-fold higher plasma concentrations than non-PMs after taking the same dose of metoprolol in a study conducted in German cardiovascular patients [30]. Moreover, CYP2D6 PMs were found to have higher risk of metoprolol toxicity [31, 32]. Thus, CYP2D6 genotype appeared to influence both the efficacy and toxicity of metoprolol.

Compared to metoprolol, the clinical evidence indicated that the effect of CYP2D6 genotype on carvedilol therapy was much smaller. For example, although a study in German healthy subjects identified the influence of *CYP2D6* genotype on carvedilol pharmacokinetics, but the genetic polymorphism of CYP2D6 was not found to affect the carvedilol effect, such as heart rate, blood pressure, or adverse effects [33].

2.3.1.2 ADRB1

The most important candidate gene for the pharmacogenomics of β-Blockers target might be *ADRB1*, which encodes the β1-adrenergic receptor, being the main drug target of several β-Blockers. The most two extensively investigated genetic polymorphisms of *ADRB1* are both nonsynonymous, namely, Ser49Gly (rs1801253) and Arg389Gly (rs1801253). The variant of Arg389 might enhance the coupling of the β1-adrenergic receptor to the second messenger adenylyl cyclase, leading to greater downstream signaling and stronger response to agonist binding. The downstream signaling could also be strengthened by the allele of Ser49 nevertheless via reducing the receptor internalization. Arg389 allele has been ever identified to be a disease risk factor of hypertension in large scale genome-wide association studies (GWAS) [34, 35], whereas the genotyping accuracy of such specific SNP in GWAS assay was questioned. A number of studies have demonstrated that Arg389 alone or together with Ser49 predisposed more potent inhibition of β-blocker, being demonstrated by greater decrease of blood pressure [36, 37]. For the case of metoprolol, the decrease of daytime diastolic blood pressure in subjects homozygous for Arg389 were about threefold bigger than in those having Gly389 variant [36]. However, such association was not always confirmed in some other studies [38, 39]. More importantly, the genetic variation of *ADRB1* was found to affect the clinical outcome of β-blockers. A large clinical study titled BEST conducted in reduced left ventricular ejection fraction (HFREF) patients found that bucindolol could achieve a 74% reduction of new-onset atrial fibrillation in patients homozygous for Arg389, whereas no efficacy in subjects with Gly389 [40]. Similarly, bucindolol could significantly decrease ventricular tachycardia/ventricular fibrillation in patients of Arg389 homozygotes but not in individuals carrying Gly389 [41]. Furthermore, a study indicated that Arg389 homozygotes had better outcome of bucindolol therapy in heart failure patients than those Gly389 carriers [42].

2.3.1.3 GRK4

Another important gene influencing β-blocker response is the gene of G protein-coupled receptor kinase 4 (GRK4), which has several functional nonsynonymous SNPs, including Arg65Leu(rs2960306), Ala486Val(rs1801058), and Ala142Val (rs1024323) affecting the drug intervention on salt related hypertension. The influence of GRK4 genetic variation on β-blocker therapy was well characterized in the African American Study of Kidney Disease (AASK), which found that carriers of 142Ala were more unlikely to benefit from metoprolol therapy in the male African-Americans with early hypertension and nephrosclerosis [43]. Moreover, the presence of 65Leu variant in carriers of 142Ala would further impair the response to metoprolol. Another two large clinical studies of hypertension demonstrated the function of 65Leu/142Val haplotype to diminish the atenolol efficacy [44]. Thus, it is no wonder that hypertension patients homozygous for both 65Leu and 142Ala were more likely to need more than one antihypertensive drug to manage their blood pressure [45].

2.3.2 Angiotensin-Converting Enzyme Inhibitors

2.3.2.1 ACE

Since Angiotensin-Converting Enzyme is the target of Angiotensin-Converting Enzyme Inhibitors, it reasonably becomes the candidate gene for the pharmacogenomics investigation of ACEI. There is a well characterized variant on the gene of ACE, namely, insertion (I) or deletion (D) of an Alu repeat in intron 16 (rs1799752). This polymorphism of I/D appeared to be risk factors of myocardial infarction or HTN. Better efficacy of both lisinopril and enalapril were associated with homozygotes of D allele in Malay male patients of hypertension [46]. This might be explained by the fact that the variant of D was also observed to elevate serum levels of ACE and relevant enzyme activity possibly offering higher potential for inhibition [47, 48]. Nevertheless, the angiotensin II levels seemed not to be affected by the D allele [49].

In addition to effectiveness, the polymorphism of D/I has been investigated as for its effect on the adverse effect of ACEI, such as potentially life-threatening angioedema. However, a body of evidence failed to find any meaningful association between D/I variant and angioedema [50].

2.3.2.2 AGT

As the starting component of renin-angiotensin-aldosterone (RAS) system, angiotensinogen also carries several genetic polymorphisms which modulate the response to ACEIs. A study in Chinese patients of hypertension found that patients

homozygous for -6G allele in the angiotensinogen (*AGT*) gene experienced significantly greater reduction in four indicators of ACEI efficacy including systolic blood pressure, diastolic blood pressure, pulse pressure, and mean arterial pressure than non-carriers of -6GG genotype [51]. However, SILVHIA trial performed in Swish patients with mild to moderate primary hypertension and left ventricular hypertrophy observed that subjects with -6A allele in the angiotensinogen (*AGT*) gene got better control of systolic blood pressure by taking atenolol compared to the carriers of -6GG [52]. This indicates that the influence of -6A>G allele might be drug or population-dependent. Also, another nonsynonymous SNP of Thr235Met (rs699) also influenced atenolol treatment and the carriers of 235Thr variant received greater pressure reduction than those of 235Met homozygotes [52]. The positive correlation of 235Thr allele with better blood pressure response was further demonstrated in the case of enalapril [53]. Whereas, some other studies failed to identify such genotype-phenotype association [54, 55].

2.3.2.3 SLCO1B1

ACEIs have a common side effect of cough, which is also associated with genetic polymorphism of *SLCO1B1*. OATP1B1 mediates the liver uptake of enalapril. The loss-of-function allele of 521C decreased the hepatic uptake of enalapril and subsequently increased the plasma exposure of enalapril [56]. Elevated plasma exposure of enalapril could increase the blood levels of bradykinin which was metabolized by ACE. The accumulation of bradykinin is the cause of cough. This explains why the allele of 521C is associated with increased risk of enalapril-induced cough [57].

2.3.3 Angiotensin II Receptor Blockers

2.3.3.1 REN

The conversion of angiotensinogen to angiotensin I is the rate-limiting step in the renin-angiotensin-aldosterone system and catalyzed by the enzyme renin, the coding gene of which harbors some variation influencing the efficacy of ACEI and ARB. A genetic polymorphism of C5312T in the distal enhancer region of renin gene (*REN*) was found to influence the *REN* transcription in vitro, and the variant of T elevated the transcription compared to the reference allele of C [58]. Consistently, a human study observed that CC homozygotes of C5312T led to better efficacy of valsartan [59]. This phenotype-genotype association gave us a clue that renin might be a biomarker of ARB effectiveness. Whereas, renin is affected by other intrinsic factors such as age in addition to genetic variation [60]. This may complicate the prediction value of C5312T.

2.3.3.2 CYP11B2

The enzyme of CYP11B2 is responsible to synthesize an important component of RAAS system, namely, aldosterone. Thus, its genetic variation on ARB efficacy has been investigated, for example, in SILVHIA trial. It was found that the polymorphism of -344 C/T in the *CYP11B2* gene could predispose the treatment effectiveness of irbesartan rather than atenolol in Swedish patients. The genotype of -344TT was associated with better response of irbesartan compared to the carriers of -344C allele [61]. However, a contrary trend was observed in another study of candesartan, in which the -344CC genotype was related to more pronounced response [62]. The inconsistence warrants more and larger clinical studies to confirm the influence of *CYP11B2* variant.

2.3.3.3 CYP2C9

Several ARBs are metabolized by CYP2C9, such as irbesartan, losartan, and valsartan. The *CYP2C9∗2* allele impairing the enzyme activity is well known to decrease the metabolism of warfarin and phenytoin compared to the wild type of *CYP2C9∗1*. Similarly, *CYP2C9∗1/∗2* was found to reduce the metabolism of irbesartan and subsequently enhance its blood pressure decreasing effect as indicated by a more significant reduction of the diastolic blood pressure being compared to the reference genotype of *CYP2C9∗1/∗1* [63]. Losartan is a prodrug, which is converted to its active metabolite of E-3174 by CYP2C9. It was indeed observed that *CYP2C9∗1/∗3* and *CYP2C9∗1/∗13* could reduce the formation of E-3174 compared to *CYP2C9∗1/∗1*, whereas such difference did not result in clinical relevance [64]. Valsartan is also metabolized by CYP2C19, but to a much lesser extent. Thus, the *CYP2C9* polymorphism was not expected to influence the exposure of valsartan [65].

2.4 Pharmacogenomics of Acute Myocardial Infarction

Ischemic heart disease and acute myocardial infarction is among the most life-threatening disease all over the world. So far nitroglycerin is still a first-line drug for angina pectoris and myocardial infarction although it has been used in clinics for more than one century [66].

2.4.1 *Nitroglycerin*

The vasodilation effect of nitroglycerin is primarily dependent on the conversion of nitroglycerin to nitric oxide catalyzed by the enzyme of aldehyde dehydrogenase 2 (ALDH2). There is a very important genetic variant occurring at nucleotide position of 1459, leading to an amino acid change from glutamic acid to lysine at the amino acid position of 487. This point mutation is referred to *ALDH∗2* and could reduce the metabolizing capacity of the enzyme by tenfold compared to the wild type of *ALDH∗1* [67]. More importantly, *ALDH∗2* is mostly distributed among the East Asians, who might originated from a Han Chinese founder in central China [68]. On the contrary, the allele of *∗2* is very scarce in Caucasians and Africans. The patients carrying *ALDH∗2* may not benefit from the administration of nitroglycerin since they could not efficiently metabolize nitroglycerin into nitric oxide. This was demonstrated in an animal study [69]. Both isosorbide mononitrate and isosorbide dinitrate can be alternative options to patients deficient of ALDH2 activity since the two drugs do not seem to require ALDH2 to convert themselves into nitric oxide [70].

2.5 Conclusion

Although huge amount of publication in pharmacogenomics of cardiovascular drugs is available, the wide application of pharmacogenomics testing has not been achieved. Since the cause of CVD is really complex procedure involving various genetic and environmental factors, relevant pharmacotherapy is also influenced by a lot of genetic and nongenetic factors. Thus, it is extremely difficult to identify the contribution of genetic variations to the treatment of cardiovascular drugs. Usually a large number of subjects and a long span of time are required to conduct reliable clinical trials of pharmacogenomics concerning cardiovascular drugs. Obviously, such studies are difficult to be performed in only one organization. Thus, national and even international collaboration is highly desired. Furthermore, with the fast development of new technology, such as big data, artificial intelligence, and real world data, clinical endpoints of high quality can be expected. After all, once pharmacogenomics of CVD comes into the era of wide application, it will benefit the biggest number of patients.

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Chapter 3
Pharmacogenomics of Antitumor Chemotherapeutic Agents

Zhaoqian Liu, Chenxue Mao, Xiangping Li, and Jiye Yin

Abstract At present, chemotherapy is still the most common treatment for cancer patients in the clinical application. Different chemotherapy drugs exert their antitumor activity in rapidly cycling tumor cells in diverse ways, including DNA damage agents, antimetabolites, antitumor antibiotics, hormone drugs, etc. However, the therapeutic outcomes, owing to lack of the individual difference awareness and the motivation of personalized treatment, vary greatly from person to person. Under the circumstances, it is necessary to identify specific biomarkers that could help to predict individualized difference in cancer treatment outcomes and potential side effects. To acquire a better understanding of the relation between human genetics and drug response, researchers tend to identify specific genetic variants of candidate genes that are associated with chemotherapeutic outcome. In recent years, advances in tumor pharmacogenomics have gradually revealed the genetic basis of interindividual differences in antitumor drugs' responses. Several biomarkers have been applied to clinical anticancer treatment in effort to improve patients' treatment benefits and reduce potential side effects. This chapter systematically described the research progress of pharmacogenomic discoveries in several types of chemotherapeutic drugs.

Keywords Cancer · Pharmacogenomics · Chemotherapy

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_3

3.1 Introduction

Following the accelerated speed of population aging and changes of lifestyle in China, cancer has gradually become one of the major diseases threatening people's health. According to the statistics in 2017, the incidence of cancer in China ranked the first position among the world, which accounts for about 22% of morbidity of worldwide tumors. Lung cancer, gastric cancer, liver cancer, colorectal cancer, and esophageal cancer were constantly the five leading cancer types. The occurrence and development of cancer, as a result of the interaction between genetic and environmental factors, is a complicated biological process. At present, chemotherapy, radiotherapy, and surgery are still the main treatment options for cancer.

Chemotherapy is presently the major route for tumor therapy. Several types of chemotherapeutic drugs are involved in clinical treatment of cancer, with various antitumor mechanisms like disrupting DNA structure and function, affecting nucleic acid synthesis, regulating hormone levels, and so on. Quite a few disadvantages are presented in traditional chemotherapy mode, especially the patients' response to chemotherapy varies greatly from each other. Other defects include nonspecific cytotoxicity, frequent drug resistance, and high toxic side effect. As a result of genetic diversity, an identical chemotherapeutic regimen that is suited for a specific population may not benefit another.

Tumor pharmacogenomics is the study of individual difference in drug metabolism and response at the genetic level, which is also the bases of individualized administration, aiming to screen out patient populations with better response and lower toxic side effects. The main study objects of tumor pharmacogenomics are searching for biomarkers that are associated with antineoplastic drug response, including strategies for drug metabolic enzymes, drug transporters, and drug targets. To summarize, tumor pharmacogenomics provide a core theory and evidence base for the realization of personalized medicine, which impenetrate in each therapeutic approach.

3.2 Antitumor Drugs Disrupting DNA Structure and Function

3.2.1 Irinotecan

UGT1A1 gene encodes uridine diphosphate (UDP) glucuronosyltransferase 1A1, which involves the glucuronidation pathway and converts small lipophilic molecules including bilirubin (preferred substrates) and certain drugs into water-soluble excretable metabolites.

Irinotecan (IRT) is a potent inhibitor of topoisomerase I mainly administered in metastatic colorectal cancer and pediatric sarcomas such as Ewing sarcoma and rhabdomyosarcoma. Irinotecan is a prodrug that is spontaneously converted to the

active metabolite 7-ethyl-10-hydroxy-camptothecin (SN-38) by endogenous carboxylesterase (CES) 1 and CES2. SN-38 inhibits the topoisomerase-1 complex, resulting in irreparable double-strand breaks in DNA, forcing cells to arrest in S phase and ultimately undergo cell death. Seventy percent of irinotecan was cleared by glucuronidation of UGT1A1 enzyme, which catabolized SN-38 into inactive SN-38 glucuronide (SN-38G) and excreted via the bile. Local accumulation of the active metabolite SN-38 could trigger dose-limiting toxicity of irinotecan, including neutropenia, myelosuppression, and secretory diarrhea.

Cancer patients treated with irinotecan can be categorized as "low toxicity" genotypes, which can tolerate significantly higher doses, and "high toxicity" genotypes, which are more likely to occur dosing toxicity. At present, two variants have been documented by numerous studies to exert an influence on the expression of UGT1A1. *UGT1A1*∗28 allele is a TATA box variant in the promoter region of UGT1A1, which reduces UGT1A1 transcription and enzyme activity, causing a 50-fold increase in the steady-state concentration of SN-38 in vivo. A recent meta-analysis further confirmed the clinical value of *UGT1A1*∗28 as a high-risk marker of irinotecan-induced neutropenia and diarrhea, especially in Caucasian populations. Another variant with high frequency is *UGT1A1*∗6 (rs4148323), which regulates UGT1A1 activity through gene expression. *UGT1A1*∗6 is more common in East Asian populations in whom the frequency of *UGT1A1*∗28 is lower in comparison to Europeans or African Americans [1].

For patients with homozygous *UGT1A1*∗28 risk allele, the current PGx guidelines recommend a 30% reduction in irinotecan dose [2]. And several regulatory bodies including FDA, the Royal Dutch Pharmacists Association-Pharmacogenetics Working Group, French Groupe de Pharmacologie Clinique Oncologique (GPCO-Unicancer), and French Réseau National de Pharmacogénétique Hospitalière (RNPGx) have also issued a warning to *UGT1A1*∗28 poor metabolizers, recommending to use lower doses of irinotecan to avoid adverse reactions [2].

3.2.2 Platinum Drugs

Platinum-based chemotherapy is the first-line chemotherapy for lung cancer, ovarian cancer, testicular cancer, and other tumors. Platinum drugs act as DNA damaging agents through three different pathways: alkylating DNA, cross-linking guanine, or inducing nucleotide mismatch, and exerts their cytotoxic function by interfering with replication and transcription. Adverse reactions of platinum drugs mainly include hematological toxicity, nephrotoxicity, neurotoxicity, ototoxicity, and gastrointestinal toxicity. Ototoxicity in children is particularly worrying because it could impair speech and normal development. In a clinical trial conducted by the Childhood Liver Tumors Study Group (SIOPEL-4), 50% of children treated with cisplatin for high-risk hepatoblastoma developed moderate or severe ototoxicity [3]. Other studies in children with medulloblastoma, osteosarcoma, and neuroblastoma observed that 60% of children receiving cisplatin experienced permanent hearing damage [4].

Previous pharmacogenetic studies have shown that hearing loss caused by cisplatin treatment is associated with *TPMT* loss of function alleles. Therefore, the Food and Drug Administration (FDA) had stated a special precaution on cisplatin drug labels that individuals with alleles *TPMT*∗3B and *TPMT*∗3C (∗3A is a haplotype of ∗3B and ∗3C) have a high risk of hearing loss [5]. Despite strong evidence supporting the relationship between TPMT and cisplatin in early studies, PharmGKB qualified it as a drug-gene pair of Level 3 evidence due to lack of repetitive validation. FDA also removed TPMT associated precautions from the cisplatin drug label, but the FDA recommended frequent hearing tests.

ACYP2 gene encodes an enzyme that catalyzes phosphate hydrolysis in membrane pumps. *ACYP2* is expressed in the cochlea and is critical for the development of hair cell. Several studies have reported that the genetic variation in *ACYP2* (rs1872328) is related to cisplatin-induced hearing loss [6–8].

Nucleotide excision repair (NER) is the major system for tumor cells to repair DNA damage caused by platinum, and ERCC1 is one of the main components of NER complex. ERCC1 forms a heterodimeric complex with XPF to catalyze the cleavage of platinum-damaged DNA from genomic DNA. Therefore, the increased expression of ERCC1 could enhance the activity of NER pathway, and ultimately promote the DNA repair of platinum-induced damage, leading to drug resistance in cancer cells. A lot of studies have shown that the mRNA expression level of ERCC1 in tumor tissues is related to the sensitivity of platinum-based chemotherapy in tumor patients, and the low expression of ERCC1 suggests that patients tend to be sensitive to chemotherapy. In addition, mutations affecting the expression of ERCC1 have also been reported to be associated with the efficacy of platinum-based chemotherapy [9]. Among them, rs11615 has been extensively studied, which could reduce the mRNA and protein expression levels of ERCC1, thereby reducing the DNA repair ability of tumor cells. Further validation is still needed in a larger sample of patients receiving cisplatin chemotherapy to provide evidence for the establishment of clinical guidelines.

3.3 Antitumor Drugs Affecting Nucleic Acid Synthesis

3.3.1 Thiopurine Drugs

Thiopurine methyltransferase (TPMT), a cytoplasmic enzyme found in almost all human tissues, is one of the phase II metabolic enzymes. This enzyme coordinates with S-adenosyl-l-methionine to catalyze the S-methylation of thiopurine drugs in the liver, such as azathioprine (AZA) and 6-mercaptopurine (6-MP). 6-MP is widely used for maintenance chemotherapy in patients with acute lymphoblastic leukemia (ALL). Thiopurine as a prodrug can be converted into 6-thioguanine (6-TG) which inhibits DNA and RNA synthesis (Fig. 3.1) [10]. Exposure to 6-TG may cause serious adverse reactions such as hepatotoxicity, gastrointestinal toxicity, and myelosuppression.

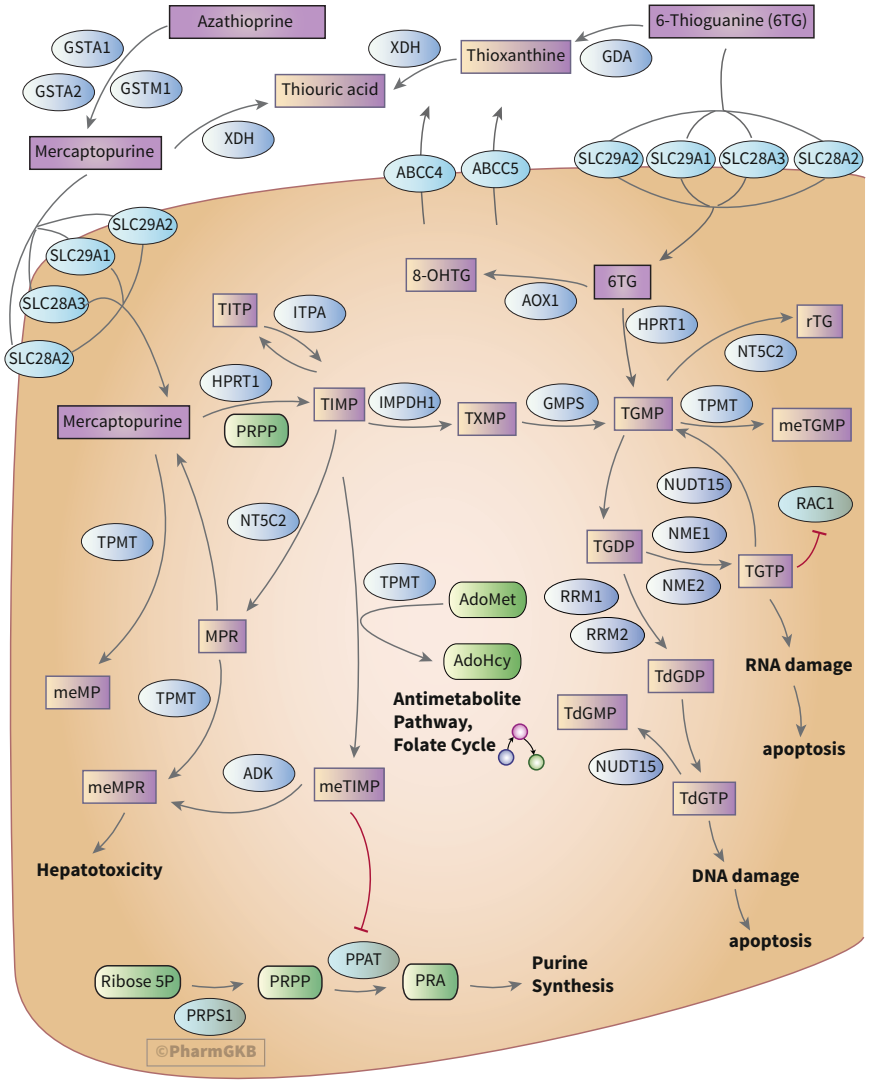

Fig. 3.1 Thiopurine pathway. (PharmGKB PA2040: https://www.pharmgkb.org/pathway/PA2040). Figure reprinted with permission from Ref. [10], copyright 2010 Wolters Kluwer Health, Inc

Polymorphisms in the *TPMT* gene can result in a dramatically decreased enzyme activity of TPMT and a higher risk of drug-induced leukopenia, and the treatment outcome of ALL patients with 6MP is highly correlated with maximum tolerable drug dose. After administration of the same dose of 6-MP, patients with different TPMT activities had a tenfold difference in steady-state drug concentrations. Thirty-three genetic variants in *TPMT* have been reported so far [11]. The most common

variant allele in Caucasians is *TPMT*∗3A with a frequency of 5%, followed by *TPMT*∗3C, which is the most common variant allele in Asians. Other common variants include *TPMT*∗2, *TPMT*∗3B, and *TPMT*∗8.

Individuals can be divided into three groups based on the absence of functional alleles: TPMT-normal metabolizers, intermediate metabolizers, and poor metabolizers. The CPIC guidelines recommend a normal dose for normal metabolizers, a 30–70% reduction of 6MP, and a 30–50% reduction of 6TG for intermediate metabolizers. Poor metabolizers receiving 6MP or 6TG should receive a 90% reduction in dose with drug administration three times per week [12].

TPMT genotyping explains part of variability in response to thiopurine therapy, but toxicities still occur in some patients with normal TPMT activity, suggesting that there are other genetic factors affecting thiopurine metabolism. A genome-wide association analysis primarily in Asian populations revealed a significant association between *NUDT15* missense mutation Arg139Cys (rs116855232, c.415C>T) and thiopurine-related myelosuppression [13].

NUDT15 is a member of nudix hydrolase superfamily, and it is homologous to nudix hydrolase 1 (MTH1) involved in the hydrolysis of 8-oxo-2′-deoxyguanosine-5′-triphosphate (8-oxo-dGTP). Patients carrying Arg139Cys homozygous risk allele of *NUDT15* gene were exquisitely sensitive to mercaptopurine, and tolerated only 8% of the standard dose. This *NUDT15* variant alone could account for 22% of the difference in mercaptopurine tolerance. However, the exact role of NUDT15 in the metabolism of thiopurine remains unclear. It was hypothesized that NUDT15 could constitute a purported purine-specific nucleotide diphosphatase, which dephosphorylates the thiopurine active metabolites TGTP and TdGTP, thus preventing their incorporation into DNA and inhibiting the cytotoxicity of thiopurine. Therefore, the thiopurine metabolism of patients with *NUDT15* variant allele is affected, with higher concentrations of active metabolites in vivo and toxic reactions due to the accumulation of metabolites. At present, the *NUDT15* genotype in Asian population has attracted much attention [14]. Inferred NUDT15 diplotypes using phased sequencing data from the 1000 Genomes Project indicated that 22.6% of East Asians were expected to be NUDT15 deficiency. South Asians were expected to be 13.6%, and Native Americans were expected to be 12%–21%. Given the relative rarity of the *TPMT* variant in Asians, more prospective clinical trials are needed to validate if NUDT15 has an important role and clinical value.

3.3.2 5-Fluorouracil

5-fluorouracil (5-FU) is the backbone of systemic combination chemotherapy for colorectal cancer. Fluorouracil analogues inhibit cell division by blocking the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). The standard dosage of 5-FU was calculated based on body surface area (BSA) of patients. However, an estimated 10–30% of treated patients develop serious 5-FU toxicity, of which 0.5–1% experiencing lethal toxicity.

The most well-known cause of 5-FU intolerance is the dihydropyrimidine dehydrogenase (DPD) deficiency. DPD encoded by the gene *DPYD* is the key rate-limiting enzyme responsible for catabolizing 5-FU to inactive 5,6-dihydrofluorouracil in the liver, and degrades up to 80–90% of an administered dose of 5-FU and capecitabine. DPD deficiency was detected in 39–61% of patients with severe 5-FU-associated toxicity [14], and DPD deficiency has been demonstrated to be linked to genetic polymorphism and leads to life-threatening early toxicity events in about 0.5% of patients receiving 5-FU treatment. The DPD enzyme activity varies greatly among individuals, with approximately 0.2–0.3% of the population with complete deficiency, and 3–5% partial deficiency, which limited the capacity of the liver to completely metabolize fluorouracil, resulting in prolongation of half-life and excessive accumulation of drugs, thus triggering toxic reactions [15].

To date, more than 120 SNPs have been published in the *DPYD* coding sequence, with the most well-established variants being *DPYD*∗2A (IVS14 + 1G> A,c.1905 + 1G> A;rs3918290) and *DPYD*∗13 (I560S,c.1679T> G; rs55886062), which were considered to have potentially deleterious impact on DPD enzyme activity. *DPYD*∗2A is a splice site variant at the intron boundary of exon 14 and results in splicing defect, skipping of the entire exon, and the subsequent loss-of-function of DPD. It is significantly related to the reduction of 5-FU degradation rate [15]. Another widely studied variant, *DPYD*∗13 (I560S, c.1679T> G; rs55886062), is very rare in the general population, with a frequency of heterozygotes of 0.2% in the white population, but it is associated with decreased DPD activity and increased incidence of toxicities.

It is known that the human genome has more than 100,000 areas of short tandem repeat sequences called microsatellites, which are susceptible to replicate "slippage" and rely heavily on MMR systems for repair. By altering the positioning of a reading frame (frameshift mutation), microsatellite length extension or reduction of the coding gene may result in the loss or acquisition of new gene function. The presence of microsatellites also affects gene expression, thereby altering both the transcription and translation processes. There are many sequences in which MSI occurs, and five sites are commonly used to determine whether MSI occurs. They are single nucleotide repeats of BAT-25 and BAT-26, and dinucleotide repeats of D5S346, D2S123, and D17S250. When two or more sites are unstable, they are defined as MSI-H, and when only one site is unstable, it is defined as MSI-L. Numerous studies have shown that the efficacy of fluorouracil in patients with MSI-H is poorer. In a study involving 570 patients, fluorouracil chemotherapy improved overall survival in patients with microsatellite-stabilized or MSI-L, whereas patients with MSI-H could not benefit from it [16].

Thymidylate synthase (TS) is considered to be the major intracellular target of fluoropyrimidine. The thymidine synthase (TYMS) gene encoding this enzyme is polymorphic, having either double (2R) or tri-tandem (3R) repeats of a 28-bp sequence in the promoter region and a 6-bp variation in the 3′-untranslated region (3′-UTR). Studies have demonstrated that *TYMS* genotyping could be helpful in predicting the toxicity to 5-FU-based chemotherapy [17], and polymorphisms in

TYMS promoter region that reduce TS expression are associated with increased fluoropyrimidine-related toxicity [18].

3.4 Antitumor Drugs Regulating Hormone Levels

In addition to antitumor drugs affecting nucleic acid, some tumors are suitable for hormone treatments by affecting endocrine, such as hormone-dependent breast cancer, prostate cancer and ovarian cancer. There are hormone receptors in these tumor cells, which depend on hormone for their growth. Therefore, drugs can be used to decrease hormone levels and block their binding to receptors, thereby inhibiting tumor progression.

Drugs affecting hormone levels can be divided into six categories, gonadotropin-releasing hormone analogues, anti-estrogen agents, anti-androgen agents, progesterone analogue, glucocorticoid and adrenal hormone synthesis inhibitor. The drugs in these classes are usually used to treat different cancers alone or in combination. Like other chemotherapeutic agents, the metabolism and clinical response of these drugs are also regulated by multiple genetic polymorphisms. Several main hormonal balancing antitumor agents and corresponding pharmacogenomics will be introduced as follows.

3.4.1 Tamoxifen

Tamoxifen is a selective estrogen receptor modulator (SERM), which was approved by the US FDA in 1977 for the treatment of metastatic breast cancer and subsequently approved for the use in the adjuvant therapy. Tamoxifen is the most commonly used drugs for the treatment of ER-positive breast cancer worldwide, while it also is a drug with a complex metabolism. A series of cytochrome P450 (CYP) enzymes catalyze the conversion of tamoxifen to its more active derivatives, namely 4-hydroxy-tamoxifen and N-desmethyltamoxifen. Thereafter, both metabolites are finally transformed into the most potent secondary metabolite of endoxifen, which has a much higher affinity towards ER (Fig. 3.2) [19]. There are more than 50 CYP genes, each of which encodes a different CYP protein product. Among these enzymes, CYP2D6 is considered the most critical enzyme of tamoxifen metabolism. Not only it is almost present in all the tamoxifen metabolic processes, but also the only isoform which converts NDM-tamoxifen into endoxifen. CYP2D6 is a highly polymorphic gene, with over 100 different polymorphisms have been described. The most commonly discussed alleles are categorized into three groups as follows: normal function (e.g., *CYP2D6*∗1 and ∗2), decreased function (e.g., *CYP2D6*∗9, ∗10, ∗17, and ∗41), and no function (e.g., *CYP2D6*∗3, ∗4, ∗5, ∗6). A commonly accepted classification designates individuals homozygous for two wild-type *CYP2D6* alleles as *CYP2D6*∗1/∗1 which is defined as extensive metabolizers

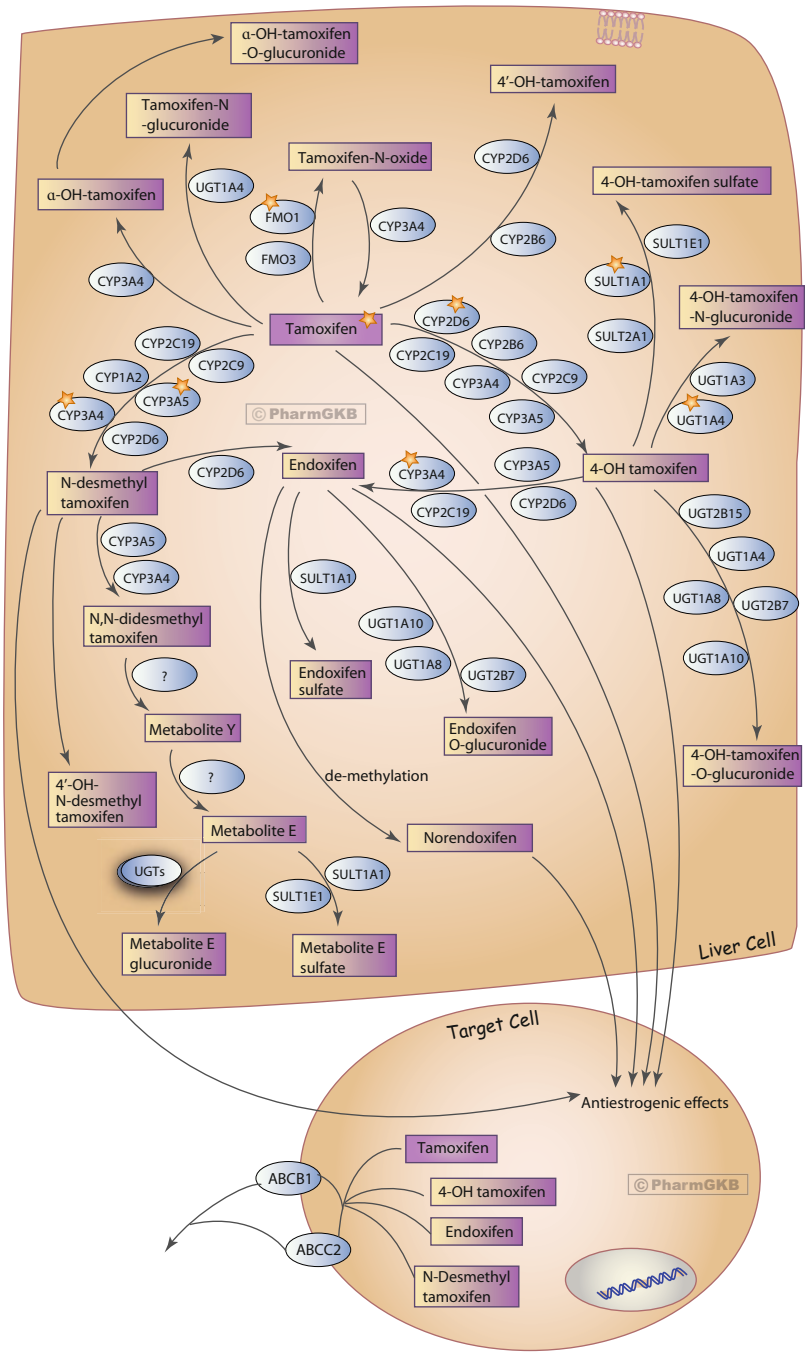

Fig. 3.2 Tamoxifen pathway, pharmacokinetics. (PharmGKB: https://www.pharmgkb.org/pathway/PA145011119). Figure reprinted with permission from Ref. [19], copyright 2013 Wolters Kluwer Health, Inc

(EMs). In contrast, individuals with one or two variant alleles that encode enzymes with reduced activity are designated intermediate metabolizers (IMs) and null activity is regarded as poor metabolizers (PMs), respectively. According to the previous reports, the most common allelic variant among Caucasians is *CYP2D6*∗4 with an allele frequency of nearly 20%, which has non-functional CYP2D6 activity [20]. Ultra-rapid metabolizers that have more than 2 normal CYP2D6 alleles are found in North Africa and Oceania. The most frequent allele among Asians is CYP2D6∗10 with decreased activity, since it is found in almost 40% of this population [21]. A prospective phase II clinical study has investigated the distribution of *CYP2D6* gene polymorphisms in Chinese population and its relationship with tamoxifen metabolism in early hormone-positive breast cancer patients. According to previous research, the frequency of *CYP2D6* variant ∗3, ∗4, ∗5, ∗6 is quite rare in Chinese populations [22]. The most common allelic genotypes of Chinese breast cancer patients are *CYP2D6*∗1, ∗2, and ∗10. The main diploids are ∗1/∗10 (38.3%) and ∗10/∗10 (18.8%). A meta-analysis showed that the blood levels of tamoxifen active metabolites HTAM (hydroxytamoxifen) in Chinese patients with *CYP2D6*∗10/∗10 genotype were significantly lower than those other genotypes ($P < 0.0001$). The patients carrying *CYP2D6*∗10/∗10 had lower blood concentration than patients with *CYP2D6* Wt/Wt genotype ($P < 0.05$). Another meta-analysis suggests that *CYP2D6*∗10/∗10 (TT) genotype is significantly associated with poorer DFS and recurrence in Asian breast cancer patients whereas has no obvious relation with overall survival [23].

3.4.2 Aromatase Inhibitors

Aromatase is an enzyme that catalyzes androstenedione and testosterone into estrogens. Aromatase inhibitors (AIs) aim to abolish aromatase activity, and block estrone/estradiol biosynthesis to slow the growth caused by estrogens. Aromatase inhibitors are commonly used in postmenopausal women with metastatic breast cancer. *CYP19A1* encodes the enzyme of aromatase and promotes the bioconversion from estrogens to androgens. Until now, there are more than 88 SNPs in *CYP19A1* with more than 44 haplotypes have been identified. Genetic variations of *CYP19A1* could have a direct impact on aromatase activity, concentrations of estrogens, side effects (such as bone loss and Vasomotor symptoms), and clinical response to AIs [24]. rs6493497/rs7176005 of *CYP19A1* has been associated with low plasma levels of estrone and low estradiol in breast cancer patients [25]. Mao et al. have found patients carrying at least one 7-repeat allele in the tetranucleotide repeat polymorphism had no significant higher risk of AAIA (AI-associated arthralgia), while carriers who have at least one 8-repeat allele usually had significant lower risk [26]. Napoli et al. reported women with ER (+) breast cancer carrying the AA genotype for the rs700518 developed significant bone loss at the lumbar spine and the total hip after 1 year treatment compared to patients carrying the G allele (GA/GG) [27]. Artigalas et al. revealed that comparing to the wild-type allele, the

presence of rs4646 G>T of *CYP19A1* was nearly two-fold increase in TTP (time to disease progression) among ER positive breast cancer patients treated with AIs [28]. Besides CYP19A1, a study in Chinese Han patients found RANKL SNP rs7984870 and OPG SNP rs2073618 were also associated with AI-related MS-AEs, and rs7984870 CC and rs2073618 CC variations were risk genotypes [29].

3.4.3 Abiraterone

Abiraterone is an oral selective CYP17 inhibitor which has obvious efficacy in combination with prednisone in both the pre- and post-chemotherapy, contributing to the increasing survival in the castration-resistant prostate cancer. The CYP17A1 is encoded by a gene located on chromosome 10q24.3 and encodes an enzyme that catalyzes key reactions in steroid hormones biosynthesis. Previous study has reported CYP17A1 expression was detected quite high in prostate cancer biopsies from patients treated with abiraterone, suggesting that upregulation of this enzyme may play a key role in the resistance to treatment [30]. Genetic variation of *CPY17A1* has been found to be associated with increased risk of prostate cancer and abiraterone responses. A clinical trial revealed 29 of the 87 patients receiving AA/P (abiraterone/prednisone) for mCRPC (castration-resistant state) harbored the rs2486758 variant of *CYP17A1* were associated with a risk for lower clinical response and shorter survival when treating with AA/P [31]. HSD3B1 is a rate-limiting enzyme necessary for dihydrotestosterone synthesis. In a trial in Japanese men treated with abiraterone, men carrying 1245C variant in *HSD3B1* gene showed lower progression risk and lower mortality risk compared with the wild type [32].

3.5 Other Chemotherapeutic Agents

In addition to the above three categories, chemotherapeutic treatment also includes the agents affecting protein synthesis and drugs intercalating DNA to interfere with RNA transcription. Among them, drugs that restraining protein synthesis can be divided into three sub-categories, L-asparaginase disturbing the raw material supply of tissue protein synthesis, harringtonine affecting ribosome function to prevent protein synthesis, the second category covers some common clinical drugs, such as colchicine, vincristine, and paclitaxel preventing tissue tubulin polymerization. The latter category includes actinomycin, daunorubicin, doxorubicin, etc. The pharmacogenomics of several important and commonly used drugs will be introduced in this section.

3.5.1 Doxorubicin

Doxorubicin is the most widely used anthracycline antibiotics chemotherapeutic agents. It is widely used for the adjuvant and palliative treatment of various malignancies, including breast cancer, sarcomas, leukemias, lung, and non-Hodgkin's and Hodgkin's lymphomas. The disposition of doxorubicin is rather complex, which involves various influx and efflux transporters and metabolizing enzymes that are responsible for the pharmacokinetics and pharmacodynamics of doxorubicin [33]. The function of SNPs in candidate gene variants related to doxorubicin disposition pathway has been investigated previously [34], including influx transporters solute carrier family (SLCs), efflux drug transporters ATP binding cassette (ABCBs), drug metabolizing enzymes carbonyl reductase (CBR) [35], and aldoketo reductase (AKR) [33, 36]. However, no consensus has yet been reached.

3.5.2 Paclitaxel

Paclitaxel is a natural alkaloid initially isolated from Taxus brevifolia, which is a widely used chemotherapy drug mainly for lung cancer, breast cancer, and ovarian cancer. It stabilizes microtubules by binding to the β-tubulin, thereby blocking cell mitosis, mediating cell death, and eventually exerting anticancer effects. Paclitaxel metabolism occurs in liver executed by CYP enzymes, namely CYP3A4, CYP3A5, and CYP2C8. Zhou et al. reported Chinese patients carrying *CYP3A5* ∗3/∗1 had a significantly higher risk of leukopenia and neutropenia than patients with *CYP3A5* ∗3/∗3. The lowest number of median leukocytes and neutrophils in patients with *CYP3A5*∗3/∗3 was significantly higher than that of patients with *CYP3A5* ∗3/∗1 [37]. Paclitaxel is also a substrate of P-glycoprotein, encoded by ABCB1, which is regarded as a main cause of paclitaxel resistance [38]. The polymorphisms of these genes have been proved to influence the metabolism, clinical efficacy, and toxicity of paclitaxel. Previous studies have reported increased paclitaxel efflux transport activity for the *ABCB1* 3435C>T variant [39]. Another research has revealed that ABCB1 3435 heterozygous patients had worse disease control ability (CC 85%, CT 50%, TT 78%) and worse overall survival (CT 13.6 vs CC 18.5 months) when compared with wild-type or homozygous variant patients [40]. Fransson et al. found that wild-type (G/G) or (A/A) patients had 30% higher clearance of 6αOH-paclitaxel than ABCB1 2677 heterozygous variant patients [41].

3.5.3 Vincristine

Vincristine is a plant alkaloid that has been utilized for treating acute lymphoblastic leukemia as well as solid tumors in adults and children. Vincristine is commonly

associated with chemotherapy-induced peripheral neuropathy (CIPN), which may occur in over 50% of vincristine-treated patients. Peripheral neuropathy is typically characterized by neuropathic pain and motor dysfunction. Vincristine is a substrate of P-glycoprotein and is metabolized by the CYP3A4 and CYP3A5, while CYP3A5 accounts for approximately 75% clearance of vincristine. Thus, alterations in CYP3A5 protein may lead to increasing vincristine toxicity. A study found that variants of *CYP3A5*∗3 and the vitamin D receptor were linked with peripheral neuropathy [42]. CEP72, encoding a protein involved in microtubule formation, has also been found to have the association with vincristine-caused peripheral neuropathy [43, 44].

3.6 Conclusion and Prospect

Although antitumor chemotherapeutic agents are widely used in clinical practice, their pharmacogenomics research is still in the early stage. Such situation points out that in order to realize the goal of personalized medicine, there is still a long way for further improvement, which needs the effort of both basic and clinical researchers. In addition, future pharmacogenomics research should not only be limited in discovery of predictive and prognostic indicators but also have to focus on critical factors that affect drug response. These will contribute to reduce toxicity and improve therapeutic effect through the regulation of these factors. Except this, there is still an urgent need for promoting the practical application of gene markers and microarrays which are instructive in individualized medicine or targeted therapy. In this way, hospitals will further initiate more detection items for drug selection, therapeutic outcome prediction, and adverse effect prevention, so as to realize standardization and routinization of tumor biomarkers detection.

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Chapter 4
Pharmacogenomics of Antitumor Targeted Agent and Immunotherapy

Zhaoqian Liu, Chenxue Mao, and Jiye Yin

Abstract Currently, cancer incidence and mortality rapidly increase and have gradually become the leading cause of death in human disease. The main medications used in clinical cancer therapy can be categorized into three types according to the pharmacological mechanism and therapeutic target, including chemotherapeutic agents, molecule-targeted agents, and immunotherapeutic agents. Targeted therapy and immunotherapy are innovative approaches in cancer therapy that has been widely accepted, both of which possess several irreplaceable advantages compared to chemotherapy. The molecule-targeted agents, which are related to higher accurate and lower toxicity, are proposed against the molecular biological targets like tumor cell proliferation, angiogenesis, apoptosis, and tumor invasion. Immunotherapy has dramatically enhanced the prognosis of tumor patients and has greatly improved the treatment for those with advanced disease. Owing to the less toxicity as well as long-term curative effect, the application of immunotherapy continues to expand with multiple new agents approved in the clinical treatment. Several pharmacogenomic biomarkers have been applied to clinical anticancer treatment in effort to strengthen the patients' treatment benefits and reduce potential side effects. This chapter systematically summarized the significant pharmacogenomic discoveries of some typical tumor therapeutic drugs involved in targeted therapy and immunotherapy.

Keywords Cancer · Pharmacogenomics · Targeted therapy · Immunotherapy

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_4

4.1 Introduction

In recent years, antitumor therapeutic strategies are developing quickly. Except chemotherapy, some emerging forms of therapeutic modalities including targeted therapy and immunotherapy are now producing a marked effect in improving tumor patients' therapy outcome.

Targeted drugs were designed on the basis of targeting definite carcinogenic sites at the cellular and molecular level. Targeted therapy, after specifically bind to carcinogenic site, induces highly selective destruction of tumor cells without affecting surrounding normal tissue. Therefore, targeted therapy provides a therapeutic method with better safety and tolerance compared to chemotherapy. However, the efficiency of molecular targeting therapy is largely dependent on the drugs' own special characteristic and the expression status of therapeutic targets in tumors. There are various targeted agents used on clinical therapeutics, such as tyrosine kinase inhibitors, anti-HER2 monoclonal antibody, BRAF kinase inhibitors, and other targeted agents. Although at current stage targeted therapy cannot completely replace the traditional treatment methods like surgery, radiotherapy, and chemotherapy, its accuracy and low toxicity provide a good prospect in the future treatment of cancer.

The progress in cancer immunotherapy highlights a promising method of cancer treatment. Cancer immunotherapy activates the immune system and recovers the dampened antitumor immune response. More narrowly, it could relieve and block cancer progression by endogenous defense mechanisms to recognize and eliminate the tumor cells and surrounding microenvironment. Identification of biomarkers being related to core immune response signal will contribute to maximize immunotherapy efficiency and boost individualized immunotherapy. Common modes of immunotherapy are PD1/PDL1 immune checkpoint inhibition and chimeric antigen receptor T-cell (CAR-T) immunotherapy, the application of which is very mature.

With the development of genetic analysis technology and pharmacogenomics, increasing number of genetic polymorphisms and mutations have been found. These genetic polymorphisms and mutations can partly explain the existence of sensitivity or resistance, as well as some severe toxic side effects. This chapter focuses on the important findings and research progress of tumor pharmacogenomics, aiming to provide insights into the potential molecular determinants of therapeutic effect of targeted and immunotherapeutic agents.

4.2 Targeted Agent

4.2.1 Tyrosine Kinase Inhibitor

Tyrosine kinase is a kind of kinase that catalyzes the transfer of gamma-phosphate from ATP to protein tyrosine residues, and can catalyze the phosphorylation of tyrosine residues in various substrate proteins, and plays an important role in cell growth, proliferation, and differentiation. Tyrosine kinase mutations are common in a variety of tumors, including colorectal, breast, ovarian, and endometrial cancers. Tyrosine kinase inhibitors have been developed as competitive inhibitors of ATP binding to tyrosine kinases and as tyrosine analogues to block tyrosine kinase activity and inhibit cell proliferation (Table 4.1). Tyrosine kinase inhibitors are highly selective with few side effects. Marketed drugs have shown their superiority in the treatment of chronic myelogenous leukemia, non-small-cell lung cancer, renal cell cancer, and many other diseases, and some of them have become the first-line drugs in the treatment of tumors. Most signal transduction inhibitors only inhibit tumor growth and cannot kill tumor cells completely. Therefore, according to current research, tyrosine kinase inhibitors should be combined with conventional chemotherapy and radiotherapy to achieve better efficacy. Although tyrosine kinase inhibitors are currently clinically effective, they also present common drug resistance and toxicity, with ethnic and individual differences.

4.2.1.1 Gefitinib

In 2004, a study found that American patients were more likely to develop resistance to gefitinib than Japanese patients. Fifteen of the 58 Japanese patients had the EGFR mutation, while only one of the 61 US patients had this mutation [1]. Some EGFR

Table 4.1 List of FDA-approved kinase inhibitors and their drug targets

Drug target	Drug
ALK	Crizotinib, ceritinib, alectinib, brigatinib
BCR–Abl	Bosutinib, dasatinib, imatinib, nilotinib, ponatinib
BTK	Ibrutinib
c-Met	Crizotinib, cabozantinib
EGFR family	Gefitinib, erlotinib, lapatinib, vandetanib, afatinib, osimertinib
JAK family	Ruxolitinib, tofacitinib
PDGFR α/β	Axitinib, gefitinib, imatinib, lenvatinib, nintedanib, pazopanib, regorafenib, sorafenib, sunitinib
RET	Vandetanib
Src family	Bosutinib, dasatinib, ponatinib, vandetanib
VEGFR family	Axitinib, lenvatinib, nintedanib, regorafenib, pazopanib, sorafenib, sunitinib

mutations (L858R, dell747-s752) [2, 3] can improve the effect of ATP-competitive inhibitors by enhancing the affinity of binding sites, while others (T790M, T854A, D761Y, L747S) [4, 5] have the opposite effect, rendering the patients with these mutations to be more susceptible to gefitinib resistance (Fig. 4.1) [7].

Abnormal expression of genes linked to the EGFR signaling cascade is the main cause of individual differences in gefitinib resistance in a variety of tumors. However, other studies have shown that EMT amplification is also an important factor to reduce the efficacy of TKIs. On the one hand, excessive activation of EMT activates the EGFR-independent PI3K pathway by up-regulating HER3. On the other hand, MET can also activate the PI3K pathway by combining hepatocyte growth factor (HGF), so that the high expression of HGF can bypass the EGFR cascade in PI3K activation, thus avoiding the inhibition of TKIs [5].

EGFR mutations lead to the tolerance to the first generation of tyrosine kinase inhibitors through a variety of biological signaling pathways, thus becoming the focus of second generation of TKIs. Neratinib, which acts on both EGFR and downstream HER2, is one of them. Recent studies have shown that the mutations of L792F, C797S, and T790M may have an impact on the efficacy of this drug, which is still controversial and needs to be further determined [8].

Among all the TKI drug-resistant patients, T790M gatekeeper mutation is the most common, with at least half of them carrying such mutations [9]. Third-generation TKIs were designed for this mutation, and the first approved drug was osimertinib. Despite the improved therapeutic efficacy of this drug, studies have suggested several mutations around the gatekeeper site (L792 position, G796D, C797S) to cause resistance to osimertinib [10, 11]. Currently, clinical experts believe that third-generation TKIs, including osimertinib, should be used as an adnexal against T790M gatekeeper, and that it is no substitute for first-generation TKIs (Fig. 4.2) [12].

4.2.1.2 Crizotinib

Crizotinib is a class of multi-target protein kinase inhibitors that can inhibit ATP competition of Met/ALK/ROS. It has been proved that crizotinib has significant clinical effects on tumor patients with abnormal ALK, ROS, and Met kinase activities. Unfortunately, like most drugs, it is beginning to develop resistance in clinical use. In 2010, a male lung cancer patient with two mutations in the ALK gene (C1156Y and L1196M) was found to be resistant to the drug. The researchers designed animal models of these two mutations and demonstrated that they reduced the sensitivity of mice to the drug [13]. In a study of 14 patients with acquired drug resistance to ALK, two recurrent mutations (L1196M and G1269A) and two additional copy number increases were found on ALK. In addition, mutations in EGFR (L858R) and KRAS (G12C and G12V) were found in these patients, suggesting that these genes may also indirectly influence the individual response to crizotinib [14]. In recent years, the researchers have understood the mechanism of drug resistance more and more deeply. The 3D modeling of ALK mutants, L1196M,

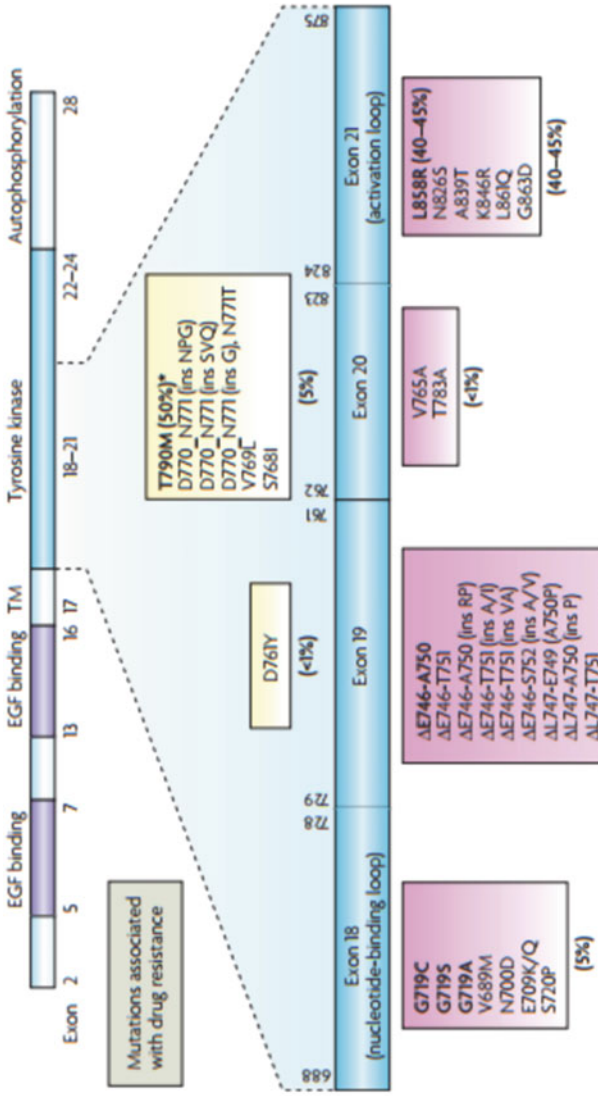

Fig. 4.1 Mutations related with efficacy of gefitinib and erlotinib in EGFR. Figure reprinted with permission from Ref. [6], copyright 2007 Springer Nature

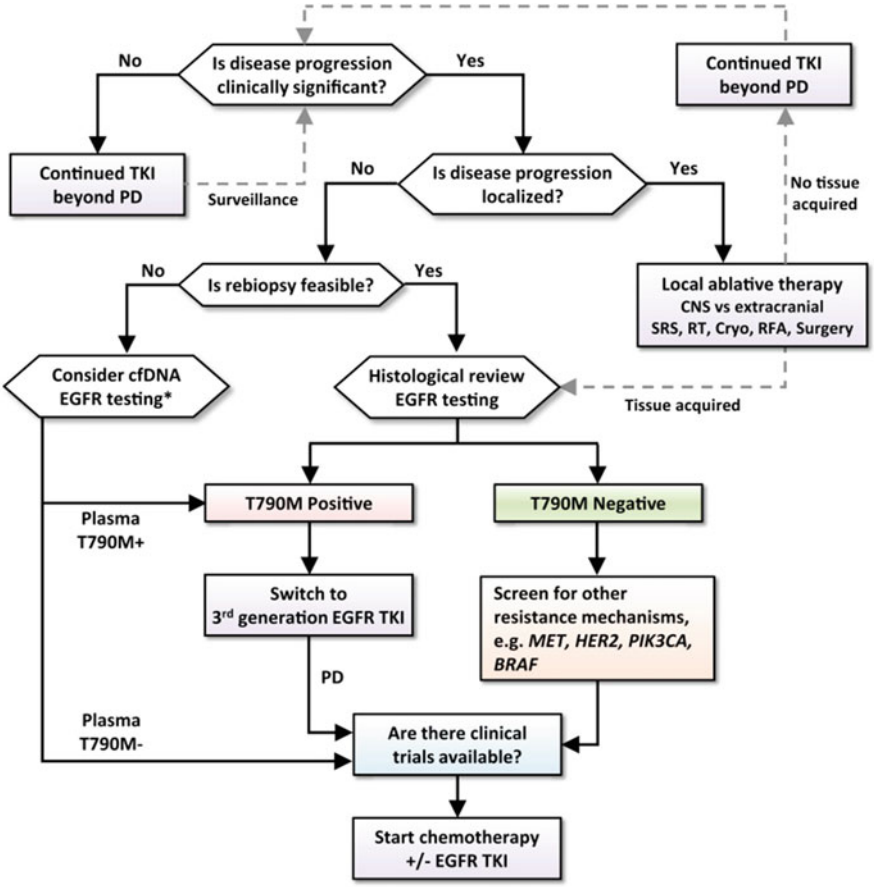

Fig. 4.2 A clinical pathway of EGFR-mutant non-small-cell lung cancer. Figure reprinted with permission from Ref. [12], copyright 2016 Elsevier

G1202R, S1206Y, and 1151 mutation surface showed obvious resistance, and the above mutants are near the crizotinib-interacting ATP-binding pocket. L1196M is believed to be a gatekeeper mutation that prevents the interaction between crizotinib and ATP-binding pocket. G1202R and S1206Y can reduce the affinity with crizotinib [15].

4.2.1.3 Imatinib

Imatinib has the function of blocking various protein kinases including tyrosine kinases. It is mainly used in the treatment of chronic myeloid leukemia (CML) and malignant gastrointestinal stromal tumors (GIST). For example, patients with mutations in exon 9 of the KIT gene had a lower sensitivity to imatinib than patients with

mutations in exon 11. Because this mutation leads to the conformation of the receptor dimer in the absence of a ligand, this may spatially hinder the binding of imatinib. In a clinical trial of 58 patients with PDGFRA D842V mutation, it was found that the mutant patients did not respond to treatment with imatinib, because the mutation of this gene would cause imatinib to fail to bind to PDGFRA [16]. Of the 31 GIST patients treated with imatinib, 15 developed secondary drug resistance and seven developed primary drug resistance. The major KIT mutations of the latter all occurred in exons 9 or 11, while the secondary KIT mutations occurred in exons 13, 14, and 17 [17]. By comparing the gene profiles of 78 patients with metastatic GIST treated with imatinib, it was found that 33 patients had secondary mutations, which also occurred in exons 13, 14, 17, and 18. Exon 14 mutations are thought to break an H-bond associated with imatinib binding, while other mutations are thought to stabilize a KIT conformation where imatinib does not bind effectively [18].

Drug resistance often leads to a relapse in CML patients, mainly due to the point mutations in the BCR-ABL kinase domain. The main reason for this resistance is thought to be the mutated subclonal amplification of the BCR-ABL protein sequence with coding changes, which prevents the binding of BCR-ABL inhibitors or forms a conformation that is less sensitive to TKIs [19]. In BCR-ABL mutations, the most common mutation regions are drug binding site and p-loop region. The mutation of 315 threonine to isoleucine (T315I) leads to the failure of H bond formation between ABL and imatinib. And the mutation leads to the changes in the conformation of several amino acid residues that reduce the drug's ability to bind to BCR-ABL. P-loop is called the induced fit site because of its conformational change accompanied by imatinib binding. Therefore, the point mutations at Y253 including Y253F and Y253H interfere with imatinib binding to Y253.

Fortunately, in recent years, both clinicians and drug development researchers are increasingly aware of the importance of precise treatment. Because certain individuals or races may carry genetic mutations that are highly resistant to their specific drug treatments, especially targeted drugs, future drug development, and clinical applications must take these drug resistance targets into account. The development of third-generation TKIs is a particularly good case in point.

4.2.2 Monoclonal Antibody Targeting EGFR

Epidermal growth factor receptor (EGFR), a cell surface protein belonging to the ERBB receptor tyrosine kinase family, actives a number of oncogenic signaling cascades, such as RAS, PI3K, and MAPK pathways, thereby regulating proliferation, angiogenesis, cell motility, metastasis, adhesion, and angiogenesis, and survival of tumor cells. EGFR is overexpressed in over 90% of tumors and correlates with poorer outcomes. EGFR status was one of the statistically significant predictors of disease-free survival (DFS). Given the function of EGFR in diverse cellular processes, this receptor tyrosine kinase has been actively pursued as a therapeutic target for cancer treatment. Therapies targeting EGFR including TKIs and

monoclonal antibodies (mAbs) are currently employed for treating patients with lung cancer, colon cancer, breast cancer, and some other types of malignancies. TKIs, including gefitinib and erlotinib, target the intracellular catalytic domain of receptor tyrosine kinases (introduced in the previous section). The monoclonal antibodies were specifically designed against the domain III of EGFR, resulting in competitive inhibition of ligand binding and preventing receptor dimerization, autophosphorylation, and downstream signaling. Currently, there are two clinically available anti-EGFR mAbs: cetuximab and panitumumab. This class of EGFR antagonists exhibited modest benefit in clinic, but the on-target toxicity limits their application, most notably skin toxicity.

4.2.2.1 Cetuximab

Cetuximab, an IgG1 human-mouse chimeric anti-EGFR monoclonal antibody, has activity against metastatic colorectal cancer (CRC) and squamous cell carcinoma of the head and neck (SCCHN) with wild-type KRAS. In addition to acting alone, cetuximab can also be used in combination with chemotherapy and radiotherapy. A phase III, multicenter trials showed that the addition of cetuximab to radiotherapy increased local-regional control and increased median OS from 29.3 months (95% CI 20.6–41.4) to 49.0 months (95% CI 32.8–69.5) for squamous cell carcinoma of the head and neck [20]. Similarly, in a phase III trial, cetuximab combined with platinum-based or 5-FU therapies increased median OS from 7.4 months to 10.1 months and progression-free survival (PFS) from 3.3 months to 5.6 months [21]. In China, the research reported that the response rates and median progression-free survivals after cetuximab treatment (PFS) in KRAS wild-type and mutant patients with colorectal cancer were 35.4% (17/48) vs. 9.1% (1/11) ($P = 0.054$) and 153 days vs. 99 days ($P = 0.01$), respectively [22]. Recently, it has been demonstrated that gene polymorphism can affect the efficiency of therapy with cetuximab, such as FCGR2A R/R and H/H genotype significantly increased the response rate [23, 24]. The AKT2:rs8100018 and the PTEN: rs12569998 homozygous variants were associated with an increased risk of progression after treatment by cetuximab [25], and AA carriers of the GC rs4588 SNP derived a survival benefit with cetuximab [26]. What is more, rs849142, located in an intron of the juxtaposed with another zinc finger protein 1 (JAZF1) gene, is associated with skin toxicity induced by cetuximab treated mCRC patients [26].

4.2.2.2 Panitumumab

Panitumumab is a fully humanized IgG2 mAb against the epidermal growth factor receptor that is approved by the US Food and Drug Administration for the treatment of metastatic CRC with wild-type KRAS. Clinical trials have demonstrated that panitumumab alone or in combination with other reagents may be efficient to improve cancer patients' survivals. Most clinical studies of panitumumab have

focused on westerners. Toshihiko et al. investigated the safety and pharmacokinetics of panitumumab in Japanese patients with advanced solid tumors [27]. Recently, a randomized phase III study of erlotinib plus bevacizumab and panitumumab is an efficient second-line treatment option for Chinese patients with non-small-cell lung cancer [28]. For efficacy of panitumumab in clinic, the somatic mutation of KRAS and NRAS was the accepted molecular marker and can be used to predict the response to panitumumab [29]. In addition, EGFR gene polymorphism of rs1050171 can affect the clinical efficacy of anti-EGFR independent of RAS mutation status [30].

The use of anti-EGFR mAbs for the treatment of cancer has significantly improved the prognosis in recent decades. However, these therapies are not able to completely cure the patients due to the drug resistance and low response rate. A better understanding of the molecular mechanisms involved in drug resistance and response rates could establish therapies to overcome resistance. Currently, decades of research have revealed multiple mechanisms modulating EGFR signals and novel EGFR monoclonal antibodies are being developed, which could help overcome the resistance and improve prognosis. In the future, continued genomic research will contribute to understand the aberrant and co-activated pathways after EGFR mAbs treatment and improve patient selection as well as future EGFR-targeted strategies.

4.2.3 Anti-HER2 Monoclonal Antibody

The human epidermal growth factor receptor (HER) is a large transmembrane glycoprotein with ligate-induced tyrosine protein kinase activity. Members of this family include HER1, HER2, HER3, and HER4. The common characteristics of HER receptor are that it contains an extracellular (EC) ligand binding region, a single transmembrane region consisting of two repetitive cysteine-rich regions, and intracellular sequences containing tyrosine kinase and autophosphorylation sites. After binding to ligands, the receptor dimerizes, which is crucial for changing the high affinity state between ligands and receptors and for the receptor to transmit phosphorylation signals between molecules. Whether a homodimer or a heterodimer is formed depends on the relative levels of the four receptors and the ligands activated. The receptor, once combined with epidermal growth factor (EGF), turns on genes in the nucleus that promote cell division and proliferation. In a variety of tumors including gastric cancer, breast cancer, bladder cancer, and squamous cell carcinoma of the head and neck, HER is overexpressed. After being stimulated by the outside world, the components of HER family can inhibit the apoptosis of tumor cells by activating downstream biological signaling pathways, such as RAS/RAF/MAPK and PI3K/AKT axis, and enhance the proliferation and invasion ability of tumor cells, thus leading to the enhancement of the malignant degree of cancer. Currently, four HER2 monoclonal antibodies have been approved by FDA for adjuvant therapy, neoadjuvant therapy, and metastatic therapy for tumors, especially breast cancer (Table 4.2).

Table 4.2 List of FDA-approved anti-HER2 monoclonal antibody

Drug	Adaptation disease	Time to market
Trastuzumab	Metastatic HER2 positive breast cancer Gastric cancer	2010
Pertuzumab	Metastatic or locally advanced HER2 positive breast cancer	2012
Trastuzumab emtansine	Metastatic or locally advanced HER2 positive breast cancer	2013
Trastuzumab-dkst	Metastatic breast cancer Gastric cancer	2017

4.2.3.1 Trastuzumab

Trastuzumab is the first monoclonal antibody developed to target HER2. The results of various clinical trials show that this drug can significantly improve the survival of patients with HER2-positive breast cancer, and it is the first-line drug to treat this kind of breast cancer. The most important mechanism leading to trastuzumab resistance in patients is the reduced ability to bind HER2, which is caused by the low expression of HER2 in the body and the mutation of HER2. Numerous studies have shown that the expression of HER2 protein level is positively correlated with the dependence of tumor development on HER2 signal, which directly affects the efficacy of drugs. Clinical trials have shown that patients with high HER2 expression are more sensitive to trastuzumab treatment, regardless of the early or late stages of disease progression. The heterogeneous expression of HER2 within the tumor may also lead to the decreased efficacy of trastuzumab-based neoadjuvant chemotherapy in patients. This is due to the competitive advantage of the HER2-negative cells in patients during the treatment process, leading to increased malignancy of the tumor [31]. In addition, the abnormal expression of HER2 splicing variants will also lead to impaired binding ability of trastuzumab to HER2. The most studied variant is the mutation of p95HER2 isoform and HER2Δ16 [32].

Changes in the activation state of signaling pathway in vivo are also one of the important factors leading to trastuzumab resistance. Since trastuzumab acts primarily as an anticancer pathway by inhibiting the PI3K/AKT/mTOR signaling cascade, there is no doubt that abnormal activation of this signaling pathway leads to drug resistance. For example, E545 K and H1047R activating mutations of PI3K catalytic subunit of alpha (PI3KCA) can lead to drug resistance of breast cancer patients [33]. PTEN is a tumor-inhibiting phosphatase that reverses PI3K-induced phosphorylation of inositide. Studies have shown that low expression of PTEN also leads to reduced ability of trastuzumab to inhibit cancer [34]. Other factors affecting drug resistance were also identified, including activation of cyclind1-cyclin-dependent kinase 4/6 (CDK 4/6) axis and abnormal expression of ER and FASN [35, 36].

4.2.3.2 Pertuzumab

Pertuzumab can combine HER2 extracellular domain II area, to block the HER2 and HER3 heterologous dimers, thus slow down cancer growth. The synergistic effect of this drug combined with trastuzumab is 50% effective in patients with the first progression of trastuzumab treatment [37], which can be used as the first-line treatment option for patients with HER2-positive advanced breast cancer. The mechanism of resistance to pertuzumab is not yet clear. In vitro studies suggested that pertuzumab could promote the rapid dimerization of HER3 and EGFR as well as the downstream pathway transduction. In addition, increased expression of p95-HER2, decreased expression of HER2, and PIK3CA activation mutation were all correlated with drug resistance of pertuzumab.

Since FDA approval and use in recent years, HER2 monoclonal antibodies have significantly improved the prognosis of patients with HER2-positive breast cancer. Although these drugs have achieved certain efficacy, drug resistance caused by the single application of targeted drugs has also severely limited their clinical use. The current multi-targeted drug combination therapy strategy has been proved helpful to improve the prognosis of patients, which has certain prospects. For various drug resistance mechanisms mentioned above, the current clinical strategy is to combine anti-HER2 MoAbs with various targeted drugs, such as inhibitors of ER, PIK3CA/mTOR, or FGFR1. Although this approach can improve efficacy to some extent, it also puts patients at greater risk of adverse reactions. Therefore, understanding the mechanism of drug resistance in HER2 targeted therapy is conducive to the development of more targeted drugs and the development of reasonable treatment plans, and the implementation of individualized and accurate treatment for breast cancer patients.

4.2.4 BRAF and MEK Inhibition

MAPK signaling pathway, a key regulator of normal cellular growth and proliferation, is a highly conserved family of protein kinases to mediate the transmission of extracellular signals to intracellular signals. In this signaling pathway, RAF first leads to the activation of MEK by phosphorylating MEK, and subsequent extracellular signal-regulated kinase (ERK) was activated. Mutations of RAF and MEK induced constitutive activation of the MAPK pathway, resulting in a tenfold greater protein activity than wild-type. This suggests that targeting BRAF or MEK may inhibit the growth of tumor and improve progression-free survival. Nowadays, selective inhibition of the MAPK pathway with either BRAF or MEK inhibition has emerged as a key component for the treatment of tumor in clinic.

4.2.4.1 BRAF Inhibitor

BRAF, a member of the family of RAF serine/threonine kinase, is a central component of the RAS–RAF–MEK–ERK signaling pathway, and regulates cell proliferation, differentiation, and survival. Aberrant activation of BRAF mutations is observed to be common in numerous cancers, such as melanomas, papillary thyroid cancers, colon cancers, non-small-cell lung cancers (NSCLCs), and leukemias. In China, the rate of BRAF mutation is approximately 25% in patients with melanoma and it serves as independent adverse prognostic factors [38]. The most frequent mutation (over 90%) of BRAF, the substitution of glutamic acid by valine at amino acid 600 (V600E mutation), results in several-fold kinase hyperactivation and participates the activation of the downstream MEK/ERK pathway, evasion of senescence and apoptosis, unchecked replicative potential, angiogenesis, tissue invasion, metastasis, and the evasion of immune response by phosphorylating multiple substrates both in the cytosol and in the nucleus (Fig. 4.3) [39]. On the basis of the essential role of BRAF mutation in the survival pathway, the novel small molecule inhibitors of BRAF were approved by FDA such as vemurafenib and dabrafenib, and elicited remarkable responses in clinic especially for melanoma.

Vemurafenib, a selective ATP-competitive inhibitor of oncogenic BRAF V600 kinase, was approved to treat metastatic and unresectable melanomas with BRAF V600E or brain metastases of melanoma by the USA in 2011. In China, vemurafenib was approved in 2017 as targeted therapies for metastatic melanoma and put into

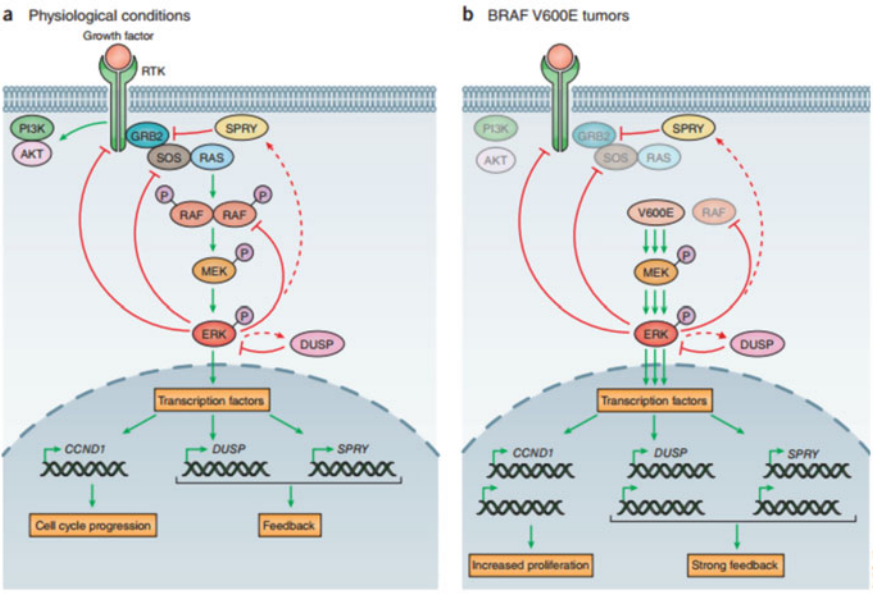

Fig. 4.3 ERK signaling under physiologic conditions and in tumors harboring BRAF V600E mutations. Figure reprinted with permission from Ref. [39], copyright 2013 Springer Nature

Chinese Guidelines on the Diagnosis and Treatment of Melanoma. The clinical trial (NCT01910181) in Chinese patients showed that vemurafenib improved progression-free survival (PFS) and overall survival (OS) compared with dacarbazine in the treatment of naive patients with BRAFV600 mutation-positive metastatic melanoma [40]. The phase I of vemurafenib showed a favorable benefit-risk profile among Chinese patients. All patients ($n = 46$) were included in the efficacy analysis population. At clinical cutoff, one patient (2%) had confirmed complete response and 23 patients (50%) had confirmed partial response. Additionally, 21 patients (46%) had best response of stable disease. The efficacy of vemurafenib in Chinese patients was generally consistent with those reported in Caucasian patients [38]. Unfortunately, acquired drug resistance to vemurafenib was observed frequently after months of therapy and resulting in disease progression [41]. Many resistance mechanisms have been demonstrated to converge in the reactivation of the MAPK pathway such as mutations of NRAS, NF1, and MEK, increased expression of c-RAF or BRAF, and altered BRAF splicing. In addition, receptor tyrosine kinases, PI3K/AKT signal pathway, AXL, HGF/MET, PDGFR, the loss of PTEN, and the EGFR family also contribute to the resistance of vemurafenib (Fig. 4.4) [42, 43].

Dabrafenib also inhibits the mutant BRAF kinase. The clinical phase III trial (NCT01227889) demonstrated that dabrafenib significantly prolonged PFS of patients with BRAF mutation from 12 countries compared to dacarbazine, 5.1 months vs. 2.7 months, respectively [44]. Thus far, dabrafenib has not been approved in China. A phase 1 study (NCT01582997) of dabrafenib in East Asia (Japan) has shown that dabrafenib has promising clinical activity in Japanese patients with BRAF mutated malignant melanoma [45].

Encorafenib, an ATP-competitive BRAF inhibitor, could suppress several mutated forms of BRAF kinase involving V600E, V600D, and V600K mutations, and the half-life is more than 10-times longer (>30 h) than either BRAF inhibitor (e.g., dabrafenib and vemurafenib). In June 27, 2018, the FDA approved the combination of encorafenib and binimetinib (MEK inhibitor) for the treatment of non-resectable or metastatic melanoma with BRAF V600E or V600K mutations. The clinic trail (NCT01909453) demonstrated that median progression-free survival was 14.9 months in the group of encorafenib plus binimetinib, and 7.3 months in the vemurafenib group [46]. This combination would provide a new treatment option for the patients with BRAF-mutant melanoma.

4.2.4.2 MEK Inhibitor

Mitogen/extracellular signal-regulated kinase (MEK) 1/2 are integral components of MAPK signaling pathway, and is aberrantly active in malignant melanoma. Several MEK inhibitors including trametinib, cobimetinib, and binimetinib have demonstrated activity as single agents or in combination with other therapies. Trametinib was the first MEK inhibitor approved by FDA as monotherapy or in combination with BRAF inhibitor (dabrafenib) for the treatment of advanced BRAF (V600)

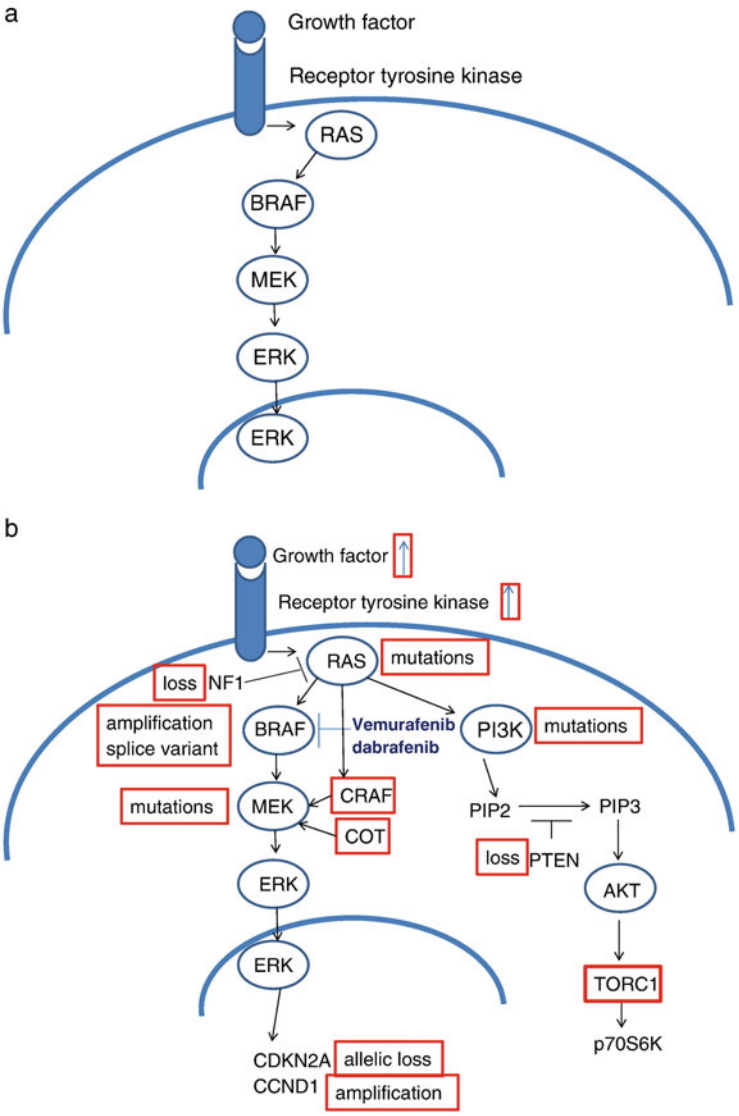

Fig. 4.4 (**a**) The MAPK pathway. (**b**) Proposed mechanisms of resistance to BRAF inhibitor therapy in the MAPK and PI3K/AKT signaling pathways. Figure reprinted with permission from Ref. [42], copyright 2014 Elsevier

mutant melanoma. A phase III clinical trial (NCT01245062) demonstrated that trametinib significantly improved response rates (22 vs. 8%), median PFS (4.8 vs. 1.5 months), and 6-month OS (81 vs. 67%) compared with chemotherapy in BRAF-mutated melanoma [47]. The most common side effects after trametinib treatment were rash, diarrhea, and peripheral edema and could be managed via dose

interruption and dose reduction. In addition, the combination of dabrafenib plus trametinib for the patients with advanced BRAFV600-mutant melanoma (NCT02296996) [48] or BRAF (V600E)-mutant metastatic non-small-cell lung cancer (NCT01336634) [49] was undergoing clinical trials and has shown efficacy. Thus far, cobimetinib was approved by the US FDA in November 2015 for the use in combination with vemurafenib for unresectable or metastatic melanoma with a BRAFV600E or V600K mutation.

The oncoprotein BRAF and MEK is a validated therapeutic target in a large number of human tumors. Currently, clinical RAF and MEK inhibitors have improved the survival of patients with melanoma whose tumors harbor BRAF mutations. However, the resistance limits their effectiveness in these patients. The future research will aim to understand the molecular and biochemical mechanisms of resistance to current clinical MAPK inhibitors, identify the optimal combination regimen for BRAF inhibitor or MEK inhibitor, and develop next-generation RAF or MEK inhibitors with high potency and selectivity.

4.2.5 Other Targeted Agent

4.2.5.1 mTOR Inhibitor

The PI3K/AKT/mTOR pathway is in disorder in many cancers including kidney and pancreatic cancer. The hyperactivation of mammalian target of rapamycin (mTOR) pathway by phosphatidylinositol-3-kinase (P13K)/Akt has been shown to be implicated in the regulation of a variety of cellular processes, including growth, proliferation, and protein synthesis. Thus, inhibiting mTOR pathway was developed as a tool in the management of patients with cancers and elicited promising strategy. Rapamycin (sirolimus) is a macrolide immunosuppressant that inhibits the mechanistic target of rapamycin (mTOR) protein kinase and widely used in antitumor and organ rejection after transplantation. Rechem et al. demonstrate that homozygous SNP-A482 of the lysine tridemethylase gene KDM4A/JMJD2A increased rapamycin sensitivity using an unbiased drug screening against 87 preclinical and clinical compounds [50]. This data provided a candidate biomarker to achieve better targeted therapy for tumor.

Everolimus, an oral inhibitor of mTOR, was approved in China to treat advanced kidney and pancreas cancer. A phase 1b study (NCT01152801) evaluated the safety and efficacy of everolimus in VEGFr-TKI-refractory Chinese patients with metastatic renal cell carcinoma [51]. In addition, as an immunosuppressant, mTOR3162/rs2295080 CC variant increased the risk of diabetes mellitus than AA or AC genotype carriers following the administration of everolimus in liver transplant recipients [52].

4.2.5.2 HDAC Inhibitor

In recent years, epigenetic regulation of gene expression is regarded as an important factor in various physiological process including cancer, immune deficits, and neurodegenerative diseases. In this context, histone deacetylase (HDACs) is currently considered as highly promising targets and HDAC inhibitors elicited promising therapeutics in the treatment of cancer and other age-associated chronic disorders. HDAC inhibitors are pleiotropic drugs, and could simultaneously target multiple signaling pathways involving BCL-2, CD95, HSP-90, HIF1α, and VEGF to suppress tumor-cell growth [53]. Vorinostat is the first approved HDAC inhibitor by FDA for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma. At present, the phase IB clinical trial of vorinostat combined with ixabepilone in the treatment of breast cancer is ongoing, and offers a novel therapy for previously treated MBC patients [54]. Romidepsin is an HDAC inhibitor approved by the FDA for the treatment of cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma (PTCL). It is recommended by the NCCN as second-line. Belinostat, an HDAC inhibitor, was approved in 2014 by FDA for the treatment of relapsed or refractory peripheral T-cell lymphoma. The phase II study in relapsed or refractory peripheral T-cell lymphoma has shown that the overall response rate in the 120 evaluable patients was 25.8% (31 of 120), including 13 complete (10.8%) and 18 partial responses (15%). Median progression-free and overall survival were 1.6 and 7.9 months, respectively. The most common grade 3–4 adverse events were anemia (10.8%), thrombocytopenia (7%), dyspnea (6.2%), and neutropenia (6.2%). Treatment-emergent AEs (TEAEs) occurred in 96.9% of patients such as nausea (41.9%), fatigue (37.2%), pyrexia (34.9%), and thrombocytopenia (16.3%) [53].

4.2.5.3 PI3Kδ Inhibitor

The phosphatidylinositol-3-kinase (PI3K) pathway contributes to multiple cellular functions, including proliferation, differentiation, and trafficking. The deregulation of PI3K was observed in many malignancies. Inhibition of the phosphatidylinositol-3-kinase (PI3K) pathway as an anticancer therapeutic strategy has drawn public attention in the last few years. Idelalisib (CAL-101, GS-1101) is an orally bioavailable ATP-competitive kinase inhibitor that targets the hyperactivated phosphoinositide 3-kinase p110 isoform δ (PI3Kδ), and was approved in 2014 in the USA and European Union for the treatment of indolent B-cell neoplasms such as relapsed/refractory chronic lymphocytic leukemia (CLL, in combination with rituximab), relapsed follicular lymphoma, and relapsed small lymphocytic lymphoma (as monotherapy). Furthermore, it was approved in the European Union as first-line therapy for CLL of poor prognosis.

In addition, other target drugs are also being used in the clinic, such as BCL-2 inhibitor ABT-199 used to treat leukemia because of its high potency and selectivity.

The emergence of targeted drugs provides an optimistic prospect for antitumor. More and more targeted drugs have been approved by all over the world. Significantly increased small molecule inhibitor or combination is currently investigated in animal or clinical trials and has shown promising anticancer effects. It is believed that some of them would be approved in the near future and provide a new treatment option for cancer patients.

4.3 Immunotherapy

The concept of cancer immunotherapy did not appear until the last few decades. It is a therapy for cancer treatment which stimulates the immune system's innate ability that is often suppressed in the tumor microenvironment (TME) by manipulating the components of the immune system. With our increasing understanding of the immune escape mechanisms that cancer has evolved, different forms of immunotherapy have been developed, including cancer vaccines, immune checkpoint inhibitors (ICIs), and chimeric antigen receptor T cells (CAR-T). Comparing to traditional therapy against cancer, these emerging classes of immune agents show great power. Some patients can dramatically benefit from immunotherapy in the case of melanoma and other forms of cancer. However, the fact that not all patients respond to immunotherapy should also be kept in mind. It is necessary to find out what factors affect patients' response to these agents and can serve as potential biomarkers to predict efficacy of immunotherapy. This section focuses on recently reported potential biomarkers for two categories of cancer immunotherapy, immune checkpoint blockers and cellular CAR-T immunotherapy.

4.3.1 PD-1/PD-L1 Immune Checkpoint Inhibitors

Recent advances in immune checkpoint blockers targeting programmed cell death/ligand-1 (PD-1/PD-L1) have profoundly changed the treatment of cancer. Monoclonal antibody drugs to block PD-1 and PD-L1 can help to release the immune brakes and unleash antitumor immune responses. PD-1 is an important immunosuppressive member of the CD28/CTLA-4 receptor family and widely expressed on the surface of multiple immune cells including T cells, macrophages, and B cells. PD-L1 is the ligand of PD-1 and mainly locates on the surface of tumor cells, dendritic cells, and macrophages. The PD-1/PD-L1 signaling pathway is activated once they are attached to each other, leading to the inactivation of T cells and immune escape of tumor cells. PD-1 inhibitors can prevent the binding of PD-1 to its ligand and restore the antitumor activity of T cells by specifically targeting PD-1 on the immune cell surface, while PD-L1 inhibitors target the PD-L1 ligand to block the PD-1/PD-L1 axis and derepress immune activity. Based on their antitumor efficacy in patients as evidenced by a series of clinical trials, PD-1/PD-L1 inhibitors have been approved

for the treatment of different solid tumors including melanoma, renal cell carcinoma, non-small-cell lung cancer, Hodgkin lymphoma, head and neck cancer, bladder cancer, liver cancer, gastric cancer, and colorectal cancer.

Currently, FDA has approved several PD-1 inhibitors (nivolumab, pembrolizumab, and cemiplimab-rwlc) and PD-L1 inhibitors (atezolizumab, durvalumab, and avelumab). Besides these PD-1/PD-L1 blockers, toripalimab and sintilimab are two domestic PD-1 inhibitors of China and their cost is much lower for patients. As effective as PD-1/PD-L1 blockers can be, the response rate to single anti-PD-L1/PD-1 antibodies is no more than 40% [55]. The unpredictability of efficacy and high cost have impeded the clinical use of ICIs. Therefore, it is of great significance to identify predictive markers of ICIs efficacy and select patient subpopulations who will benefit from ICIs.

4.3.1.1 PD-L1 Expression

Overexpression of PD-L1 is a strategy used by many tumor cells to evade immune responses [56]. PD-L1 expression on cell surface has been established as an effective biomarker in predicting the response to anti-PD-1/PD-L1 agents and approved by FDA for pembrolizumab efficacy prediction. The Keynote-001 phase 1 trial showed that PD-L1 expression (assessed by immunohistochemistry) in at least 50% of tumor cells correlated with better response to pembrolizumab in patients with advanced non-small-cell lung cancer (NSCLC) [57]. In attempts to compare pembrolizumab with standard chemotherapy, improved efficacy of pembrolizumab was observed in patients with strong PD-L1 expression [58, 59]. According to a meta-analysis enrolling 20 trials and 1475 patients with either melanoma, lung cancer, or genitourinary cancer, overall response rate (ORR) for nivolumab and pembrolizumab was significantly higher in PD-L1 positive than in PD-L1 negative patients [60]. However, other trials in renal cell carcinoma (RCC) and NSCLC showed that patients could benefit from PD-1/PD-L1 inhibitors such as nivolumab and atezolizumab, irrespective of their expression of PD-L1 [61, 62]. Therefore, PD-L1 negative tumors can also respond to PD-1/PD-L1 inhibitors, suggesting that PD-L1 expression as a predictive marker for ICIs efficacy warrants further evaluation.

These inconsistent results can be explained by several possible reasons. Firstly, different methods and platforms for PD-L1 detection make it hard to develop a consistent standard to measure the expression of PD-L1. As a result, the definition of PD-L1 expression differs among studies. Secondly, PD-L1 expression is characterized by spatial and temporal heterogeneity, which means biopsy-based PD-L1 expression cannot reflect the overall expression of PD-L1 on tumors [63]. Thirdly, both tumor cells and immune cells express PD-L1, tumoral PD-L1 expression is hard to measure accurately without excluding non-tumoral PD-L1 expression.

4.3.1.2 Tumor Mutation Burden and Neoantigens

Tumor mutation burden (TMB) refers to the number of non-synonymous somatic point mutations in the genome of tumor cells. Because a non-synonymous mutation will lead to the production of an abnormal protein, tumors with high TMB often express plenty of abnormal proteins, which can be recognized as neoantigens by the immune system [64]. The national comprehensive cancer network (NCCN) guidelines have recommended TMB as a biomarker to select patients who will benefit from immunotherapy. In a retrospective study to evaluate the effect of TMB on the response of patients with different cancers to PD-1/PD-L1 blockers, patients with higher TMB showed higher response rates and had longer progression-free survival [65]. According to the results of another study including NSCLC patients treated with anti-PD-1/PD-L1 inhibitors, higher TMB was significantly correlated with a durable clinical benefit [66]. Although TMB proves to be a promising biomarker, several problems remain unsettled. Whole-exome sequencing (WES) is the golden standard to calculate TMB, but its high cost and time-consumingness hinders its clinical use. Moreover, there is no consensus on how to define TMB cutoff.

Neoantigens can be used as a biomarker to predict response to anti-PD-1/PD-L1 therapies. Gains in PFS were observed in NSCLS patients with a high neoantigen burden after receiving PD-1 blockade treatment [67]. Based on neoantigen presentation and recognition, a fitness model that can predict tumor response to ICIs has been built [68].

4.3.1.3 Tumor-Infiltrating Lymphocytes

Tumor-infiltrating lymphocytes (TILs) refer to lymphocytes that have infiltrated into the tumor site. For tumor cells to evade immune attack, a prerequisite is that TILs can penetrate the tumor site and induce the expression of PD-L1 on tumor cells by secreting IFN-γ. However, for immune cells to kill tumors, they also have to access the tumor site. Therefore, TILs enriched in the tumor microenvironment are essential for PD-1/PD-L1 blockers to restore the local suppressed immune response. For that reason, TILs enrichment is a potential biomarker for ICIs efficacy prediction. In patients with metastatic NSCLC or metastatic melanoma, a high CD8+/CD4+ TILs ratio was correlated with a high response rate to anti PD-1 treatment [69].

4.3.1.4 Mismatch Repair Deficiency and High Microsatellite Instability

Defects of mismatch repair (MMR) pathways in tumor cells will lead to the accumulation of replication errors and somatic mutations, and are associated with microsatellite instability (MSI) and with high TMB. MMR deficiency has been reported to make cancers sensitive to PD-1 blockade, irrespective of cancer types [70]. Tumors with high MSI are characterized by lymphocytic infiltration, somatic

hypermutation, and an increased number of neoantigens. Based on a high MSI or deficient MMR phenotype of tumors, pembrolizumab was approved by FDA for the treatment of unresectable or metastatic solid tumors, regardless of their origins [71].

4.3.1.5 Gene Mutation

Genetic mutation of several genes has been identified as indicators for reduced efficacy of anti-PD-1/PD-L1 therapies. EGFR mutations and ALK rearrangements are correlated with the resistance to PD-L1/PD-1 blockers in NSCLC patients [72]. STK11/LKB1 alterations prove to be a biomarker of negative PD-L1 expression and lower response rates to PD-1/PD-L1 inhibitors in KRAS mutation-positive lung adenocarcinomas [73]. For tumors with oncogenic drivers, targeted therapy is a better choice.

4.3.2 Cytotoxic T Lymphocyte Antigen-4 (CTLA-4) Immune Checkpoint Inhibitors

CTLA-4 is expressed by both CD4+ and CD8+ T cells. Although sharing the same ligands with its homologous receptor CD28 that coordinates in T cell co-stimulation, CTLA-4 mediates opposing functions in T cell activation. By competitively binding CD80 and CD86, it blocks CD28 receptor-involved co-stimulatory signal and results in immunosuppression. Generally, CTLA-4 expression is tightly regulated to avoid both autoimmune diseases due to low expression and tumorigenesis caused by high expression-induced immunosuppressive and tumor permissive microenvironment. Increased CTLA-4 expression was observed in several cancers such as B-cell chronic lymphocytic leukemia [74]. Targeting CTLA-4 immune checkpoint, in addition to PD-1/PD-L1, is another immunotherapy strategy for cancer treatment. Ipilimumab was the first FDA-approved anti-CTLA-4 antibody to treat metastatic melanoma.

To find out which patient subpopulation will benefit more from anti-CTLA-4 treatment, increasing efforts have been put to identify factors that affect efficacy of CTLA-4 blocking agents. To date, TMB was believed as a promising biomarker for CTLA-4 blockade efficacy [75, 76]. For example, overall TMB, neoantigen burden, and cytolytic markers expression were significantly associated with clinical benefit in metastatic melanoma [76]. High serum ANGPT2 before treatment was found associated with reduced response and overall survival in metastatic melanoma patients treated with CTLA-4 and PD-1 blockers [77]. A prognostic model based on the neutrophil to lymphocyte ratio and lactate dehydrogenase may predict patients' overall survival [78]. Indoleamine 2,3-dioxygenase (IDO) is immunosuppressive, which can inhibit the antitumor efficacy of ipilimumab. Co-inhibition of IDO and CTLA-4 can markedly delay B16 melanoma growth. A high level of IL-8 at

baseline was associated with worse prognosis regardless of treatment. Serum cytokine levels have recently been reported as predictive biomarkers of ipilimumab efficacy in small-cell lung cancer [79]. Baseline elevated IL-2 levels are associated with increased response to ipilimumab, while high IL-6 and TNF-alpha levels predict resistance. An increase in IL-4 levels predicts a better overall survival [79].

4.3.3 Chimeric Antigen Receptor T-Cell (CAR-T) Immunotherapy

Chimeric antigen receptor (CAR) T-cell therapy is a live cell modality that extracts T cells from a patient's blood and genetically modifies them to express a CAR. After returning to the donor patient's blood, these modified T cells are able to recognize and kill tumor cells [80]. CAR-T cell therapy has shown unexampled response rates in stubborn leukemia. Tisagenlecleucel (CTL019) and axicabtagene ciloleucel (KTE-C19) are two CAR-T cell therapies approved by FDA in 2017 for the treatment of recurrent or refractory B-cell lymphoblastic leukemia (B-ALL) and diffuse large B cell lymphoma, respectively. Biochemical parameters such as a CD27+CD45RO+ phonotype in the CD8+ T cell population have been suggested as predictive biomarkers for the response to CAR-T cell therapies [81].

4.4 Conclusion and Prospect

Individualized diagnosis and treatment of tumors is one of the significant progresses in basic and clinical research. It provides a new idea and approach for tumor therapy. Doctors and pharmacists, as a result of making comprehensive judgement according to the genetic detection results and clinical diagnostic data, can maximize therapeutic effectiveness, avoid invalid treatment, and prolong the survival time. To sum up, with the development of tumor molecular biology and the decipherment of human genome sequence, the role of pharmacogenomics in new drug research will be far beyond our imagination. It is entirely possible for doctors to establish more personalized therapy for patients based on their genotype.

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Chapter 5
Pharmacogenomics of Immunosuppressants

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Abstract Long-term survival of patients after solid organ transplantation mainly depends on the rational use of immunosuppressants, including the calcineurin inhibitors (e.g., cyclosporine A and tacrolimus) and antimetabolic drugs (e.g., mycophenolic acid). These drugs are characterized by narrow therapeutic index, large interindividual and individual variabilities in pharmacokinetics and pharmacodynamics, promoting an urgent for therapeutic drug monitoring and individualized therapy. The pharmacokinetic variabilities can be partly explained by the genetic polymorphisms of the transporter and metabolic enzyme genes, such as cytochrome P450 (CYP) 3A4/5 polymorphisms for calcineurin inhibitors and UDP glucuronosyltransferase (UGT) 1A9 genetic polymorphisms for mycophenolic acid. In recent years, genetic polymorphisms in pharmacodynamics of immunosuppressants have been paid increasing attention. Monitoring of these pharmacogenetic biomarkers provides us a powerful approach to develop individualized dosage regimen for the immunosuppressants.

Keywords Pharmacogenomics · Immunosuppressants · Cyclosporine A · Tacrolimus · Mycophenolic acid

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_5

5.1 Introduction

One of the challenges in successful transplantation outcome is adapting of immunosuppressant therapy to the particular requirements of individual transplant patient to improve efficacy and minimize toxicity. Therapeutic drug monitoring (TDM) of immunosuppressive agents is routinely implemented to maintain the drug exposure within a defined therapeutic range. However, TDM is not useful for designing optimal starting regimen since it is only conducted after receiving immunotherapy. In addition, TDM does not provide any explanation of the underlying factors that affect the pharmacokinetics and pharmacodynamics of immunosuppressants.

Genetic polymorphisms in metabolizing enzymes, transporters, and drug targets are potential biomarkers to be used in establishing a pharmacogenetic approach to individualize pharmacotherapy. Deep understanding of the impact of genetic factors on the pharmacokinetics and pharmacodynamics of the immunosuppressants allows the determination of the optimal immunosuppressant combination for an individual patient, assisting in designing the precise starting and maintenance dosing regimen. It also helps to identify patients with increased risk of inefficacy, adverse effects, or toxicities.

At present, calcineurin inhibitors such as cyclosporine A (CsA) and tacrolimus (TAC) and antimetabolite drug (mycophenolic acid, MPA) are widely used immunosuppressive agents for the management of transplant recipients. In this chapter, a summary of pharmacokinetics and pharmacodynamics of these drugs was provided along with in-depth description on various aspects of pharmacogenomics of these agents.

5.2 Calcineurin Inhibitors

5.2.1 Cyclosporine A

Introduced in the 1980s, CsA revolutionized the care of transplant patients through its potent inhibition of acute cellular transplant rejection. Although its use has gradually been replaced by TAC, it is currently used in approximately 10% of transplants. The metabolism and mechanism of action of CsA is shown in Fig. 5.1.

5.2.1.1 Pharmacokinetics

CsA is slowly and incompletely absorbed from the gastrointestinal tract. The absolute bioavailability of CsA is estimated to be 30% (range 10–60%). In the small intestine, P-glycoprotein (P-gp) limits the absorption of CsA by active extrusion from the enterocyte interior back into the gut lumen [1]. CsA is distributed largely outside the blood volume. The steady-state volume of distribution during

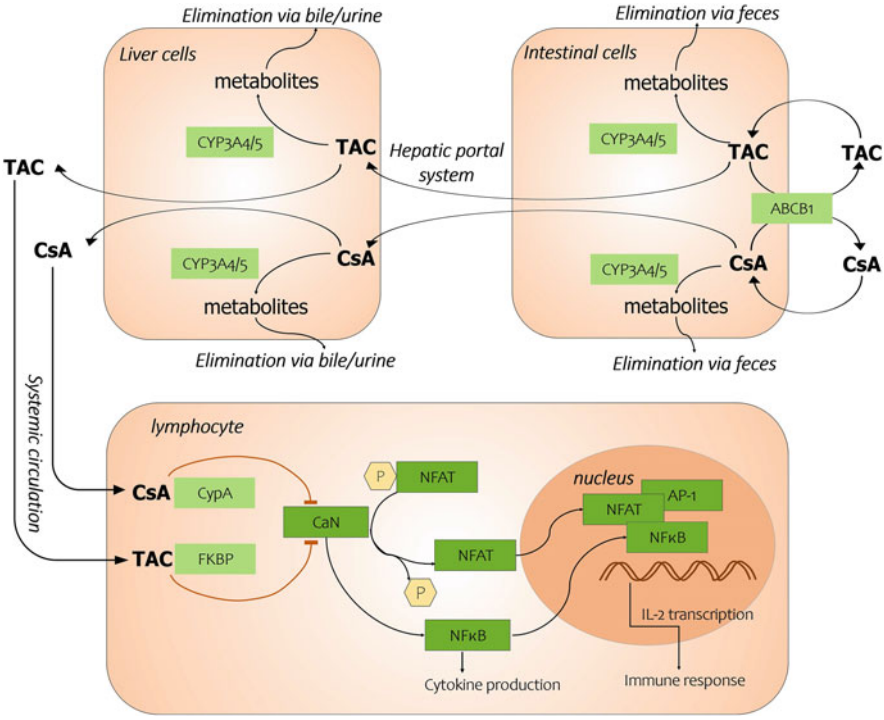

Fig. 5.1 The metabolism and mechanism of action of cyclosporine A (CsA) and tacrolimus (TAC). The oral absorption and systemic clearance of CsA and TAC is influenced by P-glycoprotein (P-gp), encoded by *ABCB1* gene (ATP-binding cassette transporter gene). CsA and TAC are metabolized by the cytochrome P450 (CYP) 3A enzymes, especially CYP3A4 and CYP3A5 in the liver and the intestine. The coding genes for CYP3A4 and CYP3A5 are CYP3A4 and CYP3A5 genes. CsA and TAC exert their actions by binding to the specific immunophilins, cyclophilin A (CypA), and FK506-binding protein (FKBP), respectively. The complex then inhibits the phosphatase activity of calcineurin, preventing it from dephosphorylating and activating the nuclear factor of activated T cells (NFAT) family. This, in turn, reduces the transcription of the NFAT-regulated gene productions, such as IL-2, which plays an important role in the immune response. CypA is encoded by PPIA gene. FKBP12 and its isoform FKBP12.6, encoded by the *FKBP1A* and *FKBP1B* genes, respectively, are notable for their combination with TAC. Calcineurin consists of a catalytic subunit (CNA) and a regulatory subunit (CNB). The PPP3CA, PPP3CB, and PPP3CC genes code for three specific isoforms of CNA (CNA-α, CNA-β, and CNA-γ, respectively). Among the three genes, PPP3CB is widely expressed in T and B cells. CNB has two isoforms, CNB1 and CNB2, which are encoded by the PPP3R1 and PPP3R2 genes, respectively. CNB1 combines with CNA-α and CNA-β, while CNB2 binds to CNA-γ. The NFAT family is a transcription factor family that contains five proteins, NFAT1–5. The NFAT proteins have a highly variable tissue distribution, with NFAT1 and NFAT2 (encoded by the *NFATC2* and *NFATC1* genes, respectively) being present in T cells and other immune cells

intravenous dosing has been reported as 3–5 L/kg in solid organ transplant recipients. In blood, CsA is extensively distributed in erythrocytes and to a lesser extent in white blood cells. In plasma, approximately 90% of CsA is bound to proteins, primarily lipoproteins [2].

The main sites of metabolism for CsA are the cytochrome P450 (CYP) 3A enzymes in the liver and the intestine. CYP3A4 may play a more dominant role than CYP3A5 in the metabolism of CsA. Three primary metabolites—AM1, AM9, and AM4N—are produced during CsA biotransformation. CYP3A4 catalyzes the formation of all three primary metabolites, whereas only AM9 is produced to any significant degree by CYP3A5 [3].

CYP oxidoreductase (POR) is a diflavin reductase that contains both flavin mononucleotide and flavin adenine dinucleotide (FAD) as cofactors and uses NADPH as an electron donor. All cytochrome P450s depend on POR for their supply of electrons for their catalytic activities to metabolize drugs, xenobiotics, and steroid hormones; therefore, alterations in POR could have significant consequences to levels of calcineurin inhibitors [4].

5.2.1.2 Pharmacokinetics Related Pharmacogenetics

CYP3A Polymorphisms

Several studies conducted in adult or pediatric renal transplant recipients found no influence of the CYP3A4∗1B allele on CsA pharmacokinetics [5–7]. In contrast, in one study conducted in 14 healthy volunteers, higher dose-normalized area under the concentration-time curve (AUC) of CsA was observed in homozygous carriers of the wild-type genotype as compared to homozygous carriers of the variant genotype [8].

The CYP3A4∗1G (rs2242480), a new single nucleotide polymorphism (SNP) in intron 10 of CYP3A4, was first found by direct sequencing in a Japanese population and is characterized by G to A substitution at position 82,266, which was incorrectly named CYP3A4∗18B in some published articles. The CYP3A4∗1G allele exists in approximately 25% of Japanese and 37–49% of Chinese subjects [9, 10]. It was speculated that this nucleotide mutant might be associated with increased CYP3A4 activity [11]. Hu et al. indicated that the CYP3A4∗18B polymorphism may be associated with increased CYP3A4 activity and is responsible for the inter-individual differences in the pharmacokinetics of CsA [12]. Qiu et al. also found the CYP3A4∗18B genotype affects CsA pharmacokinetics during the first month following surgery in Chinese renal transplant recipients. Patients with CYP3A4∗18B alleles may require higher doses of CsA to reach the target levels [9].

CYP3A4∗22 (rs35599367 C>T) is an allelic variant of the CYP3A4 associated with a decreased activity. As pharmacokinetics of CsA is concerned, Lunde I et al. found CsA C_2/D was 53% higher among CYP3A4∗22 carriers [13]. However, Cvetković M did not find any association between this variant and the pharmacokinetics of CsA in pediatric renal transplant recipients [14].

CYP3A5∗3 (rs776746, 6986A>G) is the most important SNP defined as CYP3A5∗3 located within intron 3 of the CYP3A5 gene with the wild-type allele as CYP3A5∗1. The association between the CYP3A5∗3 allele and CsA exposure has been widely studied. The CYP3A5∗3 allele was found to result in lower dose-standardized exposure to CsA in some studies which conducted in both healthy volunteers and renal transplant patients of Caucasian, Chinese, or Indian origins. However, there were also studies that found no association. The consequence of CYP3A5∗3 on CsA pharmacokinetics remains thus uncertain and may not be of major concern. A reason for this can be the limited catalytic efficiency of CYP3A5 as compared to CYP3A4 regarding CsA [15].

ABCB1 Polymorphisms

The drug transporter P-gp, which is the product of ABCB1 (ATP-binding cassette transporter gene) gene in humans, acts as a barrier of xenobiotic absorption by its efflux pump activity in the intestine [16]. A number of SNPs have been identified in the ABCB1 gene by large-scale sequencing [17, 18]. The most common investigated SNPs of ABCB1 gene are C3435T (rs1045642) in exon 26, C1236T (rs128503) in exon 12, and G2677T/A (rs2032582) in exon 21.

The influence of ABCB1 SNPs on CsA pharmacokinetics remains uncertain. Staatz et al. reviewed more than 10 independent studies, mainly conducted in renal transplant patients, and found no associations between the ABCB1 SNPs of 1236C>T, 3435C>T, or 2677G>T and the pharmacokinetics of CsA [19]. Conversely, a few studies have shown significant associations concerning 3435C>T SNP. The variant allele at position 3435 was associated with decreased exposure to CsA in 75 Caucasian renal transplant recipients [20]. However, in at least two other studies, the SNP was associated with significantly higher dose-normalized CsA exposure [21, 22]. A recent meta-analysis involving 1036 individuals (healthy volunteers or renal transplant patients) from 14 different studies also failed to demonstrate a definitive association between the 3435C>T SNP and CsA pharmacokinetics [23]. The fact that CsA is a potent inhibitor of P-gp may limit or suppress the influence of genetic polymorphisms and presumably explain part of the discrepancy reported [15].

POR Polymorphisms

POR is highly polymorphic with more than 40 variant alleles. POR∗28 (rs1057868; 1508C>T) is the most common SNP. POR∗28 encodes the amino acid variant p.A503V, which lies in the FAD binding domain. It has been shown that there was a decrease in the dose-adjusted CsA concentrations for POR∗28/∗28 homozygous individuals compared with patients carrying a POR∗1 allele. This association was seen in patients not carrying the CYP3A4∗22 (decrease-of-function allele) [4].

5.2.1.3 Pharmacodynamics

CsA derives its primary immunosuppressive activity by selectively binding to cyclophilin A, a peptidylprolyl isomerase present within the cytoplasm of cells. Once bound, the CsA/cyclophilin complex inhibits the enzymatic activity of the calcineurin. Calcineurin removes critical regulatory phosphorylation on nuclear factor of activated T cells (NFAT) triggering its translocation to the nucleus of T cells, where it synergizes with other factors to mediate the transcription [24].

5.2.1.4 Pharmacodynamics Related Pharmacogenetics

Acute Rejection

Few studies have analyzed the influence of the CYP3A4 -392A>G SNP on CsA pharmacodynamics. A retrospective analysis of 124 Caucasian renal recipients (>6 months after transplantation) could find no relationship between recipient CYP3A4 -392A>G genotype and the incidence of acute rejection or renal function as assessed by creatinine clearance [6]. Meanwhile, two studies, conducting either in 237 Caucasian renal recipients or in 67 Asian renal recipients, could find no relationship between recipient carrying CYP3A5 6986A>G genotype and the incidence of acute rejection [3, 19].

Several studies have analyzed the influence of ABCB1 3435C>T, 1236C>T, and 2677G>T/A SNPs on CsA pharmacodynamics. In 237 Caucasian renal recipients, patients who were homozygous for the variant ABCB1 2677T allele were more than twice as likely to experience biopsy-proven acute rejection (BPAR) as ABCB1 2677G homozygous patients [25].

Besides, NFATC1 rs3894049 GC was found to be a risk factor for acute rejection compared with CC carriers ($p = 0.0005$) [26].

Nephrotoxicity and Renal Function

Several studies have analyzed the influence of CYP3A5∗3 genotype on patient renal function or CsA-related nephrotoxicity; however, no relationship was found [3, 19].

Some studies have examined the relationship between the ABCB1 genotype and patient kidney function. A case-control study involving 53 German heart recipients with renal insufficiency and 53 controls found no association between recipient ABCB1 2677G>T/A genotype and susceptibility to renal insufficiency [27]. A case–control study involving 97 German renal recipients and their donors found that a significantly higher proportion of patients who received a donor kidney with the variant of ABCB1 3435TT experienced nephrotoxicity, compared with those who received a donor kidney with the ABCB1 3435CC or CT genotype [28]. Such a finding is consistent with possible lower functional activity of P-gp in subjects with

the variant genotype and potentially reduced export of drugs from kidneys cells, leading to nephrotoxicity.

FOXP3, a member of the forkhead/winged-helix family of transcriptional regulators, has been shown to be specifically expressed in naturally occurring $CD4^+CD25^+FOXP3^+$ Tregs. A study including 166 renal transplant patients treated with CsA-based immunosuppressive regimen showed patients with rs3761549 T/TT genotype had a more rapid decline in the estimated glomerular filtration rate (eGFR) level during the 5 years following transplantation than those with the C/CC genotype (24.0% vs. 6.3%, $p = 0.004$) [29].

Xu et al. investigate the effects of 76 SNPs in the cyclophilin A/CaN/NFAT pathway genes (PPIA, PPP3CB, PPP3R1, NFATC1, and NFATC2) on CsA efficacy in renal transplant recipients, and they found NFATC1 rs2280055 TT carriers had a more stable eGFR level than CC ($p = 0.0004$) [26].

Moscoso-Solorzano GT et al. identified two SNPs in the cyclophilin A gene: one located on the first exon (c.36A>G) and the other on the gene promoter (c.-11C>G). No correlation between these SNPs and acute rejection was found, whereas the SNP -11C>G was associated with an increased risk of nephrotoxicity (OR = 3.5; CI 95% 1.5–8.2, $p = 0.006$) [30].

5.2.2 Tacrolimus

TAC, also known as FK506, was introduced into clinical practice in 1989 as an alternative to CsA, and it achieved the approval by US Food and Drug Administration (FDA) for the use in patients following liver transplantation in 1994.

The metabolism and mechanism of action of TAC is also shown in Fig. 5.1.

5.2.2.1 Pharmacokinetics

TAC is absorbed rapidly in most subjects, with peak plasma/blood concentrations obtained at 0.5–1 h. Absorption of TAC from the gastrointestinal tract after oral administration is incomplete and variable. The poor aqueous solubility of TAC and alterations in gut motility in transplant patients may be partially responsible for poor and erratic drug uptake. As with CsA, TAC is also the substrate of P-gp, which may limit the absorption of TAC in the small intestine [1]. The average bioavailability of TAC is merely 25%, and it varies dramatically among individuals, ranging from 5% to 90%. About 99% of TAC binds to erythrocytes after entering the systemic circulation, but only the dissociated portion can enter the lymphatic system and play its major immunosuppressive effect. Blood drug concentrations are significantly higher than the corresponding plasma values [31, 32].

TAC is extensively metabolized by CYP3A4 and CYP3A5 in the small intestine, liver, and kidney. CYP3A5 may play a more dominant role than CYP3A4 in the metabolism of TAC in individuals who are CYP3A5 expressers (CYP3A5∗1/∗1 or

∗1/∗3). The intrinsic clearance of TAC is approximately twofold higher for CYP3A5 than for CYP3A4 [3].

5.2.2.2 Pharmacokinetics Related Pharmacogenetics

CYP3A Polymorphisms

The effect of CYP3A4∗1G polymorphism on TAC pharmacokinetics has been studied by several groups. However, the functional consequence of this SNP is controversial [33, 34].

The associations of TAC pharmacokinetics and CYP3A4∗22 have been extensively explored by many researchers. Elens et al. confirmed the decreased CYP3A4 activity toward TAC for CYP3A4∗22 carriers early after transplantation [35]. In a multicenter, prospective, and randomized study conducted by Pallet N et al., they found the CYP3A4∗22 allelic variant is associated with a significantly altered TAC metabolism and carriers of this polymorphism often reach supratherapeutic concentrations [36].

For CYP3A5 expressers (CYP3A5∗1∗1 or CYP3A5∗1∗3), CYP3A5 may play a more dominant role than CYP3A4 in the metabolism of TAC. Meanwhile, the intrinsic clearance of the drug by CYP3A5 is approximately twofold higher than that by CYP3A4 [37].

A strong association between CYP3A5∗1/∗3 and TAC pharmacokinetics has been demonstrated in various studies conducted in renal or lung transplant patients. The dose required to achieve the therapeutic range in renal transplantation was estimated to be twice as much in carriers of at least one active CYP3A5 allele of ∗1 than in noncarriers with ∗3 [15]. In liver transplant patients, the mean TAC C_0/dose was lower in recipients engrafted with a liver carrying the CYP3A5∗1/∗1 genotype [38]. These findings are consistent with experimental evidence that CYP3A5 contributes significantly to the metabolic clearance of TAC in the liver and kidney [37]. In a prospective multicenter clinical trial which is aimed to determine CYP3A5 genotype as the biomarker in TAC initial dose adjustment, renal transplant patients ($n = 280$) were randomly assigned to receive TAC at an initial dose either based on the CYP3A5 genotype or according to the recommended daily regimen. Further dose adjustments based on TAC C_0 were allowed in both arms. After six doses, a significantly higher proportion of patients had reached the therapeutic range in the adapted group than in the control group (43.2% vs. 29.1%, $p = 0.030$). In addition, the number of dose modifications was significantly less in the adapted group (280 vs. 420 over 3 months; $p = 0.004$) [39].

ABCB1 Polymorphisms

Regarding the relationship between ABCB1 polymorphisms and TAC pharmacokinetics, the results are still controversial [15]. In a recent review, Staatz et al.

identified 21 studies, mainly conducted in renal transplant patients, which failed to find an association between the 3435C>T SNP and TAC pharmacokinetics. Nine negative studies concerning the 1236C>T and 15 negative for the 2677G>T/A were also listed [3]. Conversely, a few studies demonstrated contradictory results that a higher or a lower dose was required in patients with the ABCB1 3435TT variant genotype [40].

POR Polymorphisms

Several studies have shown that CYP3A5 activity is affected by POR∗28 in TAC-treated kidney transplant recipients. A gain of CYP3A5 activity has been linked to the POR∗28 genotype in kidney transplant recipients expressing CYP3A5 and carrying at least one POR∗28 variant allele, and they showed significantly lower TAC exposure early post-transplantation [41]. This is consistent with previous observations that POR∗28 is associated with increased early TAC dose requirements in patients carrying a CYP3A5∗1 allele [42]. Because this effect was only observed in patients expressing CYP3A5, it suggests that POR∗28 may have a more significant effect on CYP3A5 activity with respect to TAC metabolism [4].

5.2.2.3 Pharmacodynamics

TAC exerts its immunosuppression effect by acting on FKBP-CaN-NFAT pathway. The binding of TAC to FKBP inhibits the phosphatase activity of calcineurin, preventing the dephosphorylating and activating of nuclear factor of activated T cells (NFAT) family. This can reduce the transcription of the NFAT-regulated gene productions, such as IL-2 [43, 44], which plays an important role in the immune response [45]. Theoretically, polymorphisms in each of these different proteins may affect the cellular response to calcineurin inhibitors. For instance, it was shown that mutations generated by site-directed mutagenesis in calcineurin were associated with decreased effect of CsA or TAC [15].

5.2.2.4 Pharmacodynamics Related Pharmacogenetics

Acute Rejection

The genetics basis associated with acute rejection has been extensively studied, showing that SNPs of CYP3A and ABCB1 were not the risk factors of rejection [32]. Despite a strong association between the CYP3A5∗3 polymorphism and the pharmacokinetics of TAC, there is no consistent evidence for organ rejection as a result of genotype-related under-immunosuppression. Studies could find no relationship between recipient with CYP3A5∗3 genotype and BPAR [46].

Cytokines are key mediators in the induction and effector phases of all immune and inflammatory responses. SNPs in cytokines and their receptors may relate to acute rejection. Chen et al. investigated the relation between acute rejection and SNPs in the genes encoding for IL-2 (-330G>T), IL-10 (-592C>A and -1082G>A), TGF-β1 (915G>C), and IL-2RB (rs228942 C>A and rs228953 C>T) in 325 renal transplant patients taking TAC or CsA as immunosuppressive therapy. However, they found that no statistically significant associations between the SNPs studied and acute rejection [47].

Nephrotoxicity and Renal Function

Studies on the relationship between CYP3A5 genotype and the risk of TAC-induced nephrotoxicity have reported contradictory results. Some groups have proved that CYP3A5 expressers have an increased risk for biopsy-proven TAC-induced nephrotoxicity while a Chinese study including 67 kidney recipients showed a higher incidence of nephrotoxicity in CYP3A5 non-expressers (CYP3A5∗3/∗3) at 1 month post-transplant [48–50]. In a study with 136 renal transplant recipients, those with the CYP3A5∗3/∗3 genotype also tended to have a higher incidence of biopsy-proven nephrotoxicity compared to carriers of CYP3A5∗1 allele, although the difference was not significant [51]. There are many reasons for these discrepancies, including differences in ethnicity, sample size, and the definition of nephrotoxicity [52]. Several studies have analyzed the influence of ABCB1 SNPs of 3435C>T, 1236C>T, and 2677G>T/A on the pharmacodynamics of TAC. However, few have successfully shown a relationship [46].

Qiu et al. detected in 114 Chinese renal transplant patients and demonstrated the TAC-induced acute nephrotoxicity was associated with FOXP3 rs3761548 polymorphism in renal transplant patients [53]. They recently also investigated the potential impact of FKBP/CaN/NFAT signaling pathway SNPs on efficacy and safety of TAC and finally found that the patients with FKBP1A rs6041749 TT genotype had a more stable eGFR level than CC and CT carriers ($p = 2.08 \times 10^{-8}$) during the 2 years following transplantation [54].

Besides rejection and nephrotoxicity, other clinical outcomes were found to be associated with some SNPs in transplant patients. FKBP5 rs136780, rs9296158, and rs9470080 are related to depression in patients taking TAC [55]. Chen et al. observed a relationship between rs10141896T allele of NFATC4 gene expressed in pancreatic islets and lower risk of new-onset diabetes after transplantation in Hispanic kidney transplant patients [56]. Besides, NFATC4 rs10141896 is lately shown to have no relationship with graft rejection [57].

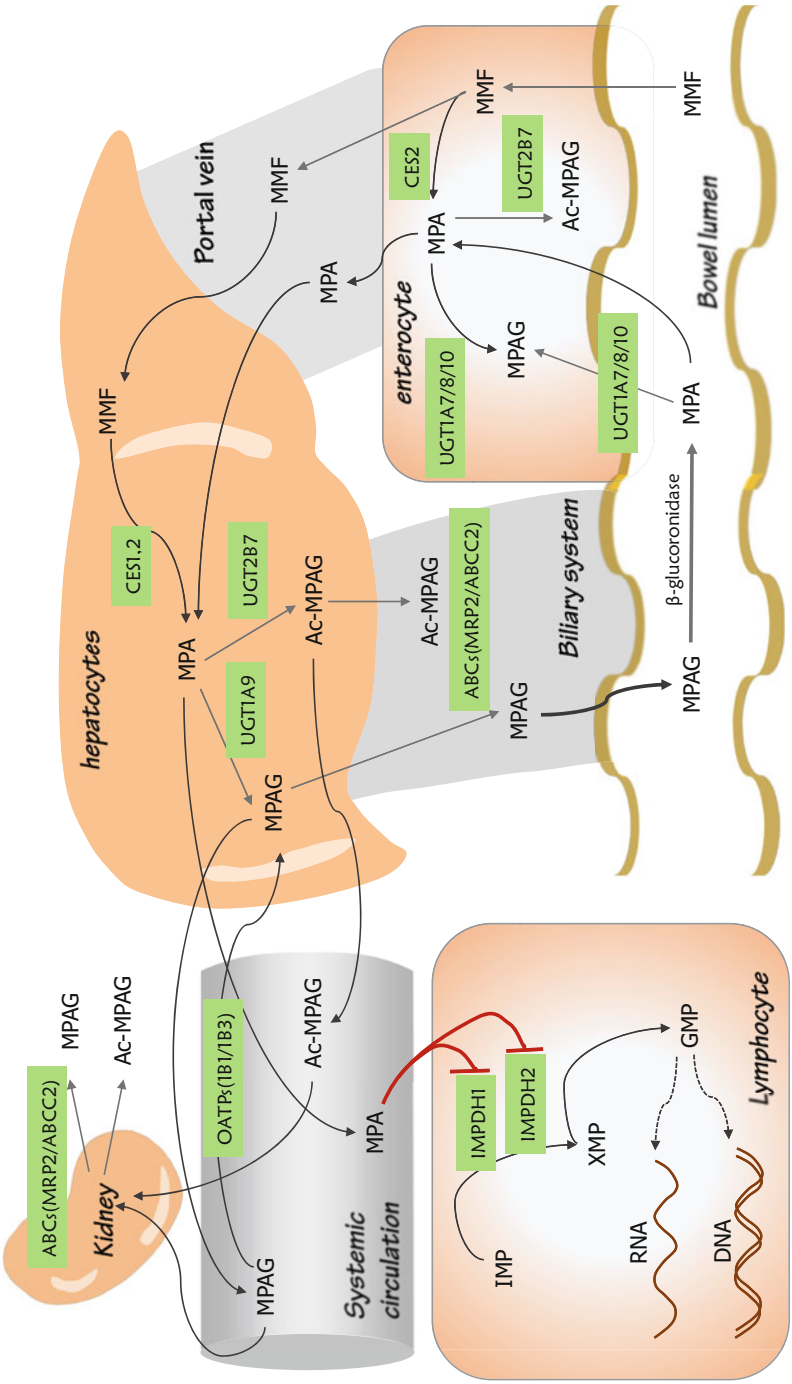

Fig. 5.2 The metabolism and mechanism of action of mycophenolic acid. ABC, ATP-binding cassette; ABCC2, ATP-binding cassette, subfamily C, member 2; Ac-MPAG, acyl glucuronide MPA; CES, carboxylesterase; EC-MPS, enteric-coated sodium salt; GI, gastrointestinal; GMP, guanosine monophosphate; IMP,

5.3 Mycophenolic Acid

MPA was first approved for preventing allograft rejection in 1995 by FDA. It has become the predominant antimetabolite immunosuppressant drug after solid organ transplantation as well as in the management of autoimmune diseases [58–61]. The metabolism and mechanism of action of MPA is shown in Fig. 5.2.

5.3.1 Pharmacokinetics

MPA is currently used both as an ester prodrug (mycophenolicate acid mofetil, MMF, CellCept®) or an enteric-coated sodium salt (EC-MPS, Myfortic®). Previous studies [62–65] have demonstrated that MMF of 1000 mg twice daily and EC-MPS of 720 mg twice daily showed similar efficacy and safety profiles, and drug exposure reflected by AUC of MPA [62, 64, 65].

After oral administration, MMF and EC-MPS are extensively hydrolyzed to active component MPA. MMF hydrolysis is mainly catalyzed by carboxylesterase (CES) 1, predominantly expressed in the liver and CES2 in the intestine [66]. In whole blood, 99.99% of MPA is found in the plasma with only 0.01% in cellular elements [67]. MPA and the main metabolite 7-O-mycophenolic acid glucuronide (MPAG) are about 97–99% and 82% bound to human serum albumin (HSA), respectively, in patients with normal kidney and liver function [68, 69].

In humans, the primary metabolic pathway of MPA is glucuronidation, occurring mainly in the liver, as well as in the kidney and the intestine. MPA is primarily metabolized by UDP glucuronosyltransferase (UGT) 1A family to form the abundant but inactive metabolite MPAG. UGT 1A9 is the most efficient for MPAG synthesis and apparently responsible for 55%, 75%, and 50% of MPAG production by the liver, kidney, and intestinal mucosa, respectively [70, 71]. MPAG is also formed by UGT 1A7, 1A8, and 1A10, which are expressed in the kidney and gastrointestinal tract [71].

Acyl-glucuronide mycophenolic acid (AcMPAG), a minor metabolite of MPA, is produced by UGT 2B7 [71], which shows a comparable inhibitory effect on lymphocyte proliferation and might be responsible for some of the adverse effects such as leukopenia or gastrointestinal toxicity [72].

The metabolite of MPAG undergoes enterohepatic circulation through biliary excretion, intestinal deglucuronidation by the gut flora, and then reabsorption as MPA. The process is likely to be mediated by UGT, organic anion transporting polypeptides (OATP), and multidrug resistance-associated protein 2 (MRP2) [73]. It has been reported that enterohepatic circulation contributes to approximately 40% of

Fig. 5.2 (continued) inosine 5′-monophosphate; IMPDH, inosine 5′-monophosphate dehydrogenase; MMF, mycophenolate mofetil; MRP2, multidrug resistance-associated protein 2; MPA, mycophenolic acid; MPAG, MPA-7-O-glucuronide; OATP, organic anion transporting polypeptides; UGT, UDP glucuronosyltransferase; XMP, xanthine 5′-monophosphate

MPA AUC [74, 75]. Over 90% of the administered dose is excreted in the urine, mostly as MPAG (87%) [69].

5.3.2 Pharmacokinetics Related Pharmacogenomics

5.3.2.1 Carboxylesterase Polymorphisms

The study conducted in 80 adult Japanese kidney transplant recipients has demonstrated that CES2 polymorphisms showed no significant influence on MPA pharmacokinetics and incidence of acute rejection or diarrhea [76]. Additionally, to the best of our knowledge, the impact of genetic polymorphisms in CES1 on MPA pharmacokinetics has not been assessed.

5.3.2.2 UGT Polymorphisms

Based on evolutionary divergence, the mammalian UGT superfamily can be divided into two families (UGT1 and UGT2) and further into three subfamilies (UGT1A, UGT2A, and UGT2B) [77]. The human UGT1A family members locate on chromosome 2q37 and contain multiple unique first exons, as well as the conserved exons 2–5, which give rise to nine types of functional UGT1A isoforms (UGT1A1, UGT1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, and 1A10) and four types of nonfunctional isoforms (UGT1A2, 1A11, 1A12, and 1A13). The UGT2A and UGT2B genes lie on chromosome 4q13, and encode three (UGT2A1, 2A2, and 2A3) and seven functional proteins (UGT2B4, 2B7, 2B10, 2B11, 2B15, 2B17, and 2B28) [78]. Among these functional polymorphisms, UGT 1A9 and 2B7 are believed to be the key isoforms responsible for the formation of MPAG and AcMPAG, respectively. Additionally, UGT1A8, mainly located in the gastrointestinal tract, has also been involved in the metabolism and first-pass effect of MPA [73].

5.3.2.3 UGT1A9 Polymorphisms

The impact of the two common SNPs in UGT1A9 promoter (-275T>A and/or -2152C>T) is widely investigated in clinical studies and probably more clinically pertinent [73, 79–88]. But the mechanism associated with the change in MPA pharmacokinetics remains unclear. Eight of the eleven studies showed significant associations between MPA pharmacokinetics and genetic polymorphisms of UGT1A9-275T>A/-2152C>T. Only three did not [83, 84, 87]. However, these two SNPs are rare in Asian.

Kuypers et al. found that MPA $AUC_{0-12\ h}$, $AUC_{0-6\ h}$ and the estimate of enterohepatic circulation ($AUC_{0-6\ h}/AUC_{0-12\ h}$) in -275T>A and/or -2152C>T

carriers were significantly lower than those of noncarriers in 95 white kidney transplant recipients [73]. However, this effect was dose dependent and only observed in patients given 2 g/day of MMF. Another study conducted in 100 white kidney recipients illustrated a significantly higher proportion (34.62% vs. 23.51%) of MPA $AUC_{0-12\ h}$ was below the recommended target range (30–60 mg·h/L) in patients carrying one or both of -275T>A and -2152C>T SNPs [88]. Other three studies conducted in kidney transplant recipients showed that carriers of -275T>A and/or -2152C>T displayed a reduction of 20–30% in MPA $AUC_{0-12\ h}$ [81, 82, 85]. Moreover, a study conducted in 51 Caucasian lung transplant recipients showed that -275TT carriers showed higher MPA dose-normalized trough concentrations than -275TA carriers [86].

Other two widely investigated SNPs in UGT1A9 promoter are -440C>T/-331T>C [83, 89–94]. Three of the above-mentioned studies showed no significant associations between MPA pharmacokinetics and genetic polymorphisms in UGT1A9 -440C>T /-331T>C [83, 90, 94], whereas the other four studies did not.

Several studies [73, 79, 82] have reported a significant increase in MPA dose-normalized $AUC_{0-12\ h}$ in subjects carrying the UGT1A9∗3 allele (98T>C, rs72551330). Additionally, the AUC of AcMPAG was also found to increase by 85% in carriers of UGT1A9∗3 [79]. Johnson et al. reported that carriers of UGT1A9∗3 had higher dose-corrected trough concentrations in comparison to noncarriers [80]. In contrast, Ruschel et al. [95] reported that there were no associations between UGT1A9∗3 allele polymorphism and exposure of MPA and MPAG. Nonetheless, UGT1A9∗3 allele is very rare, and the aforementioned studies did not have a sufficient power to reach reliable conclusion.

5.3.2.4 UGT1A8 Polymorphisms

To the best of our knowledge, only the impacts of UGT1A8∗2 ($A^{173}G$, 518C>G) and UGT1A8∗3 (830G>A) on MPA pharmacokinetics have been reported. At least 11 studies have investigated the effect of the UGT1A8∗2 on MPA pharmacokinetics [79, 80, 82, 84, 93, 94, 96–100], and the associations between UGT1A8∗2 allele and MPA pharmacokinetics were found in four studies [80, 82, 93, 98].

Johnson et al. showed MPA dose-normalized trough concentrations were about 60% higher in subjects heterozygous or homozygous for UGT1A8∗2 polymorphisms than in those with the wild-type ($p = 0.02$). However, this effect was dependent on comedication of calcineurin inhibitor and was only obvious in the TAC group ($p < 0.01$) [80]. But the study conducted by van Schaik et al. in kidney transplant recipients got an opposite result [82]. The study conducted in 127 Chinese transplant patients receiving MMF and TAC showed that the UGT1A8∗1/∗2 and ∗2/∗2 subjects were observed to present a 22% lower dose-normalized MPAG AUC_{0-12h} in comparison to the carriers of UGT1A8∗1/∗1 [93].

At least four studies have investigated the effect of UGT1A8∗3 allele on MPA pharmacokinetics [79, 80, 82, 84]. No obvious associations between UGT1A8∗3

allele and MPA pharmacokinetics were found, although there was robust evidence showed this allele decreased enzyme activity [101, 102].

5.3.2.5 UGT2B7 Polymorphisms

The allelic frequencies for UGT2B7∗1 and UGT2B7∗2 are similar (0.511 vs. 0.489) in Caucasians ($n = 91$), whereas UGT2B7∗1 is approximately threefold more prevalent than UGT2B7∗2 (0.732 vs. 0.268) in Japanese ($n = 84$) [103]. A similar allelic frequency (0.672 vs. 0.328) has been found in Chinese ($n = 218$) [104]. This ethnic diversity has been further confirmed, in which the frequencies of UGT2B7∗1 and UGT2B7∗2 are 0.463 and 0.537, respectively, for Caucasians ($n = 202$), and 0.734 and 0.266, respectively, for Asians ($n = 32$) [105].

The impact of this mutation is still controversial. Some studies have indicated that this polymorphism had little effect on enzyme activity and substrate specificity of UGT2B7 [103, 106–108]. In vitro, Bernard et al. have illustrated that the UGT2B7 enzyme generated high levels of AcMPAG with no detectable formation of MPAG, and no significant changes in the kinetic parameters were observed between UGT2B7∗1 protein and UGT2B7∗2 protein [102].

Djebli et al. have investigated the production of AcMPAG by human liver microsomes genotyped for the UGT2B7 -842G>A and 802C>T SNP which are in a perfect reverse linkage disequilibrium [109]. The -842G>A substitution was found to cause a significant increase of AcMPAG production in vitro. Similarly, an increased CL_{int} of AcMPAG production was obtained using the pool of human liver microsome (HLM) carrying one or two alleles of the -842A variant as compared with the pool of HLM carrying the wild-type genotype [109]. Given that the 802C>T coding SNP is most likely nonfunctional, these results strongly suggest that the pharmacogenetic effect is due to the -842G>A SNP.

As mentioned above, the formation of AcMPAG is a minor metabolic pathway for MPA and UGT2B7 enzyme is found to only catalyze the production of AcMPAG. Thus, it is unlikely to observe any substantial effect of UGT2B7 polymorphisms on MPA pharmacokinetics. However, Levesque et al. found that the UGT2B7∗2 allele was associated with higher free and total MPA $AUC_{0-12\ h}$ in 52 healthy volunteers received a single dose of MMF [79]. This result is surprising because the carriers of UGT2B7∗2 had no significant difference in AcMPAG levels in comparison with noncarriers.

5.3.2.6 MRP2 Polymorphisms

MRP2 (ABCC2) is considered as a main transporter involved in MPAG excretion both in the liver and in the proximal renal tubular cells and this transporter is essential for enterohepatic circulation. A number of studies investigated the impact of the ABCC2 polymorphisms on MPA pharmacokinetics [82–84, 86, 87, 89, 91,

94, 97–99, 110–114]. The -24C>T, 3972C>T, and 1249G>A SNPs were observed to be associated with MPA pharmacokinetics.

Moreover, the impact of ABCC2 -24C>T SNP might be dependent on the concomitant drug [98, 114]. In the study performed in 66 kidney transplant recipients of eight Spanish centers, MPA-reduced exposure was observed in subjects with MRP2 -24C>T variant (TT/CT: 48.12 ± 4.9, CC: 68.73 ± 6.78, $p = 0.023$) at steady-state conditions [114]. When taking into account of treatment groups, this association was only found under macrolides treatment at month 3. Controversially, Picard et al. showed that heterozygotes for MRP2 -24C>T SNP were associated with higher MPA dose-normalized $AUC_{0–12h}$ as compared with homozygotes for the reference or the variant genotype in patients who received CsA, while no significant association was found between ABCC2 genotype and MPA exposure in patients receiving TAC or sirolimus [98].

Quite a few studies investigated the effect of the ABCC2 1249G>A SNP on MPA exposure [83, 84, 86, 87, 89, 94, 110, 112, 113]. Only the study by *Zhang* et al. found that patients carrying the heterozygous mutant alleles of ABCC2 1249G>A exhibited higher dose-normalized $AUC_{6–12h}$ of AcMPAG than those with wild-type genotype ($p = 0.016$) in Chinese kidney transplant recipients [113].

5.3.2.7 OATP1B1 and OATP1B3 Polymorphisms

Picard et al. [98] investigated the role of OATP1B1, and OATP1B3 in MPA and MPAG uptake using HEK cells and reported that cells expressing OATP1B3 and OATP1B1 accumulated more MPAG. Furthermore, the authors found that the pharmacokinetics of both MPA and MPAG were significantly influenced by the OATP1B3 polymorphism 334T>G/699G>A in kidney transplant patients receiving MMF in combination with TAC or sirolimus ($n = 70$), but not in patients receiving MMF in combination with CsA ($n = 115$). The similar results were drawn by another study [97] in Japanese kidney transplant patients, in which the subjects homozygous for the SLCO1B3 334G/699A allele had significantly higher MPAG dose-normalized $AUC_{0–12h}$ and the ratio of MPAG/MPA than those heterozygous or homozygous for the reference allele. Two studies have investigated the impact of OATP1B1 polymorphisms, with one by Miura et al [111] showing higher dose-normalized MPAG exposure in SLCO1B1∗1/∗1 carriers in comparison to carriers with the SLCO1B1∗15 allele ($p = 0.002$) and another by Michelon et al [115] reporting reduced MPA transport associated with the SLCO1B1∗5 allele ($p < 0.002$).

5.3.3 Pharmacodynamics

MPA is a reversible, selective, and noncompetitive inhibitor of inosine 5′-monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in de novo

synthesis of guanine nucleotides by catalyzing the conversion of inosine 5′-monophosphate (IMP) to the critical precursor xanthine 5′-monophosphate (XMP). Lymphocytes are primarily dependent on this pathway, whereas other cell types, including polymorphonuclear leucocytes and neurones, depend primarily on the alternative salvage pathway. By depleting guanosine and deoxyguanosine nucleotides preferentially in T and B cells, MPA inhibits the lymphocytes proliferation and suppressed cell-mediated immune responses and antibody formation. Additionally, MPA can also induce T-lymphocyte apoptosis [116–118].

5.3.4 Pharmacodynamics Related Pharmacogenetics

Human IMPDH is encoded as two isoforms, type 1 and type 2, locating on two different chromosomes (7q32.1 and 3p21.31) with 85% of their amino acid sequence conserved [116]. IMPDH 2, containing 14 exons encoding 514 amino acid proteins of 56 kilodaltons, is mainly expressed in activated T and B lymphocytes, whereas the type 1 isoform is constitutively expressed in most cell types [117]. Although MPA is demonstrated to inhibit the activity of both type 1 and 2 isoforms, IMPDH 2 is fivefold more sensitive to MPA inhibition in comparison with IMPDH I [118].

5.3.4.1 IMPDH 1 Polymorphisms

Two SNPs within IMPDH 1 intron 7, rs2278293 (IVS7 + 125 G>A) and rs2278294 (IVS8-106 G>A), were observed to be significantly associated with the incidence of BPAR in the first year after renal transplantation. The odds ratio (OR) by logistic regression analysis was 0.34 for SNP rs2278293 (95% CI: 0.15–0.76, $p = 0.008$) and 0.40 for rs2278294 (95% CI: 0.18–0.89, $p = 0.02$) [119]. This protective effect of SNP rs2278294 toward BPAR was confirmed by another study [120] in 456 kidney transplant patients. Compared to the wild-type, the risk of BPAR was lower in the rs2278294 variant allele carriers (OR: 0.54, 95% CI: 0.34–0.85, $p = 0.0075$). Additionally, leucopenia was also found to be significantly associated with rs2278294 SNP (OR: 1.66, 95% CI: 1.11–2.48, $p = 0.0139$). Conversely, another study [121] in Japanese kidney transplant recipients ($n = 82$) indicated there were no significant differences in the incidence of subclinical acute rejection as for rs2278293 and rs2278294 polymorphisms ($p > 0.05$).

5.3.4.2 IMPDH 2 Polymorphisms

At least five studies investigated the influence of IMPDH 2 rs11706052 SNP (IVS7 + 10T>C), also referred as to 3757T>C or 2674T>C [25, 120, 122, 123]. Two of them observed no significant impact of rs11706052 polymorphism on any clinical outcomes [119, 120]. Two studies suggested that rs11706052 SNP

might be associated with a poorer response to MPA treatment [122, 123]. Another study conducted in de novo kidney transplant patients showed that subjects carrying one or two C alleles ($n = 28$) were threefold more likely to experience BPAR than TT homozygous carriers ($n = 193$) at 3 months after transplantation, and this trend increased slightly at 12 months (OR: 3.39, 95% CI: 1.42–8.09, $p = 0.006$) [25].

5.4 Conclusion

In the past few decades, understanding of the pharmacogenomics of immunosuppressant in transplant patients has been largely improved. Polymorphisms of the genes coding for enzymes, transporters, and drug target involved in the pharmacokinetics and pharmacodynamics of immunosuppressive agents have been extensively investigated.

Among previous findings, the strong association between the CYP3A5∗3 polymorphism and TAC pharmacokinetics is well recognized and could be useful in routine clinical practice for the individualized regimen. The influence of other genetic polymorphisms still remains uncertain since the pharmacogenetic effects on the exposure and response of immunosuppressive are complex. In future, standardized clinical trials with adequate sample size that assess the relationship between individual genetic makeup and pharmacokinetics as well as pharmacodynamics are desired.

Acknowledgement The authors would like to thank Miss Xiao-Qin Liu for preparation of the figures.

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Chapter 6
Pharmacogenomics of Psychiatric Drugs

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Abstract Psychiatric pharmacogenomics combines the science of pharmacology and genetics to predict the interindividual outcomes (i.e., therapeutic response and adverse drug reactions) of psychiatric drug treatment. In the last decade, there has been an explosion in research examining the variation in therapeutic and adverse effects of antipsychotics (APs) across individuals. Current studies mainly focus on the identification of pharmacokinetic targets (e.g., cytochrome P450 superfamily) and pharmacodynamic targets (e.g., serotonin, dopamine, and norepinephrine). This chapter provides a review of relevant pharmacogenetic studies and treatments of psychiatric disorders in clinical practice. It also includes a brief discussion for future directions.

Keywords Psychiatric pharmacogenomics · Antipsychotic · Drug targets · Clinical practice · Psychiatry disorders

6.1 Introduction

Pharmacogenomics, as a part of personalized medicine, aims to determine the impact of genetic variations or polymorphisms on the interindividual differences in drug outcomes. The ultimate goal of pharmacogenomics is to predict the responses of medication and/or adverse drug reactions (ADRs) in patients. Although there is a rich study history of pharmacogenomics, the area of psychiatric pharmacogenomics is a relatively young field, and the most relevant studies have been conducted only in the last decade. Polymorphisms in genes that encode metabolic enzymes and drug targets were successfully identified to be associated with individual clinical outcomes in psychiatric pharmacogenetic studies, such as single nucleotide polymorphisms (SNPs) in *CYP2D6, CYP2C19, DRD2, DRD3, HTR1A, HTR2A, COMT,*

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_6

SLC6A4, BDNF, GNB3, FKBP5, ABCB1, etc., which possibly influence the response to APs. At present, most of medical therapies were based on the subjective judgments by clinicians. However, the first dose of most commonly prescribed medications (e.g., APs, antidepressants, and mood stabilizers) was usually ineffective or intolerant in 40–60% of patients worldwide. Thus, an innovative approach that can characterize psychiatric disorder by combining molecular science with clinical practice may promote the development of medicine in prediction, prevention, and personalization [1]. The US Food and Drug Administration (FDA) has added recommendations for pharmacogenetic testing to drug labels for commonly prescribed medications, many of which are now available in generic forms, such as selective serotonin reuptake inhibitors (SSRIs). This chapter evaluated the role of pharmacogenomics in the treatment of psychiatric disorders including schizophrenia, depression, bipolar disorder and substance abuse, and reviewed the latest studies on the identifications of the predictors in AP treatment and novel therapeutic targets via pharmacogenomics testing.

6.2 Pharmacogenomics of Schizophrenia

6.2.1 Pharmacogenetic Studies in Schizophrenia

Pharmacogenomics studies on APs were mainly aimed at finding reliable indicators of treatment responses or adverse events. Clinical psychiatric pharmacogenomic studies have shown positive associations between schizophrenia treatment outcomes and some atypical APs including risperidone, olanzapine, clozapine, etc. [2–5]. Although most studies on drug efficacy were focused on pharmacokinetics-related genes (e.g., *CYP2D6* and *CYP2C19*), case-control studies were conducted to explain the relationships between SNPs and adverse effects. Recently, one study reported that some disease-susceptibility-related variants were identified by genome-wide association study (GWAS) approaches, and polygenic scoring was considered powerful to predict the drug response and adverse events [6]. With the cumulative studies, novel analytical tools will be invented and be used in data analysis. In the future, we believe more convincing biomarkers can be found and guide clinical practice.

6.2.1.1 Efficacy of Antipsychotic Treatment

The response to APs was mainly affected by variants in genes encoding drug-metabolizing enzymes, drug-transporting enzymes, and the receptors mediating drug responses. In particular, many APs are multi-acting receptor antagonists. Thus, genes that encode for dopaminergic D2, D3, and D4 receptors (*DRD2*, *DRD3*, and *DRD4*, respectively), serotonergic receptors (*HTR2A*, *HTR2C*, and *HTR1A*) have been reported to be associated with the efficacy of APs treatment

[7]. As we know, cytochrome P450 (CYP450) enzymes are involved in the metabolism of more than 85% of drugs, and CYP450 genes that are relevant to the main metabolic pathways of APs (e.g., *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A4*) have been reported to be associated with the efficacy and plasma concentration of APs. In addition, it was reported that *GRM7*, *COMT*, *ABCB1*, *SLC1A1*, *PCDH7*, *CNTNAP5* and *TNIK* were associated with the efficacy of APs treatment [7, 8].

The relationship between SNPs and APs efficacy is not completely repeatable. Therefore, effective prediction methods of APs efficacy have been developed in clinical applications, such as multiple genetic markers, polygenic risk scores (PRSs), and rare variants [9–11]. For example, a recent study has demonstrated that the combination of six markers might be the optimal method to predict the efficacy of iloperidone in schizophrenia patients by measuring the changes of positive and negative syndrome scale (PANSS) score [9]. For patients with one of the six single-labeled genotypes, the odds rates to iloperidone therapy response that were improved for at least 20% were 2.4–3.6. For patients with a combination of six markers, the response rates appeared to increase to 9.5 or higher. These results indicated that the combination of genetic markers may be able to improve the response to iloperidone and increase the accuracy of prediction of the drug response. PRSs, on the other hand, function as a predictive score of AP efficacy by using GWAS results from multiple SNPs with small effects. Zhang et al. [9] have suggested that patients with low PRS were more likely to be good treatment responders than patients with high PRS. Furthermore, rare variants may play an important role in APs response. It is reported that rare genetic variation in glutamatergic or N-methyl-D-aspartate neurotransmission is implicated in short-term antipsychotic medication efficacy [11]. Thus, the application of pharmacogenomics may facilitate drug selection and improve the individualized treatment of schizophrenia in clinical practice [10]. PRS analysis and rare variants appeared to have the potential to be utilized in future clinical practice.

6.2.1.2 Adverse Effects of Antipsychotics

The most common adverse effects of APs in the schizophrenia patients are glucose and lipid metabolism dysfunctions. In patients with APs treatment, more than 50% of them have been reported to gain $\geq$7% of weight compared to their baseline weight, approximately 12% of them were diagnosed with type 2 diabetes, and over 30% of them have been noticed to have metabolic syndrome (MetS) [12–14]. Chlorpromazine, olanzapine, and clozapine were associated with the highest risk for weight gain, followed by amisulpride, iloperidone, paliperidone, quetiapine, and risperidone in the moderate risk, and aripiprazole, lurasidone, and ziprasidone with the lowest risk [15]. However, the underlying mechanism of AP-induced weight gain is not completely understood. Pathways through oxidative stress reactions, alternation of ghrelin and leptin release, inflammatory, and other signaling pathways (e.g., those involving dopamine, histamine, serotonin, muscarinic, cannabinoids, and

adiponectin) have been implicated as important contributors for AP-induced weight gain [16]. A recent review has shown that a wide range of variants in multiple genes (e.g., *FTO, LEP, LEPR, MTHFR, HTR2C, CNR1, BDNF, SREBF2*, *ADRA1A,* and *ADRA2A*) were associated with the MetS in schizophrenia patients [17]. Therefore, multi-omics datasets would be greatly helpful for improving our understanding of the mechanisms of MetS and alleviating MetS in psychiatric patients in the future.

6.2.2 Treatment of Schizophrenia in Clinical Practice

In recent years, commonly used atypical APs (e.g., risperidone, olanzapine, and clozapine) have been studied in a great number of pharmacogenomics studies to discover the relationships between drug response and psychotic disorders. Recent pharmacogenomic studies and clinical trials have found some evidences of pharmacogenomic-guided medication treatment improved therapeutic effects in patients; however, these evidences of biomarkers were not solid enough to guide medication treatment in clinical practice [18].

6.2.2.1 CYP and Other Genes Related to Antipsychotic Treatment Response

CYP 2D6, 3A4, and 1A2 are metabolic enzymes mainly involved in the AP metabolism (Fig. 6.1). A large number of functional variants of the coding gene of CYP 2D6, 3A4, and 1A2 have been identified and characterized [19]. Clinical phenotypes associated with CYP variants have been classified into four categories: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultrarapid metabolizers (UM). Patients with PM or UM appeared to experience poorer responses during treatment compared to others. For instance, PM patients were more likely to maintain not only a high plasma concentration of APs that promised a relatively active drug response but also a higher risk of adverse events. UM patients, on the contrary, appeared to have less adverse events and decreased therapeutic response to their treatment (Fig. 6.1) [20].

FDA-approved labels of APs have highlighted the pharmacogenomic information based on *CYP* gene specification, including the drug–drug interaction and the dosage recommendation related to *CYP* genes [20]. Special caution is required when the APs metabolized by certain *CYP* gene are co-administrated with other drugs that inhibit or induce the *CYP* gene. For example, the combination of the mood stabilizer like carbamazepine and other strong *CYP3A4* inducers has been found to decrease the plasma concentrations of risperidone according to PERSERIS™ (risperidone) drug label. Thus, modification of the dosage of each medicine is crucial in such circumstances. *CYP2D6*, the most thoroughly studied gene among all the genes in the *CYP* family, is the primary metabolizer in the commonly applied antipsychotics [19]. The dosage guidelines according to *CYP2D6* phenotype (particularly for PM

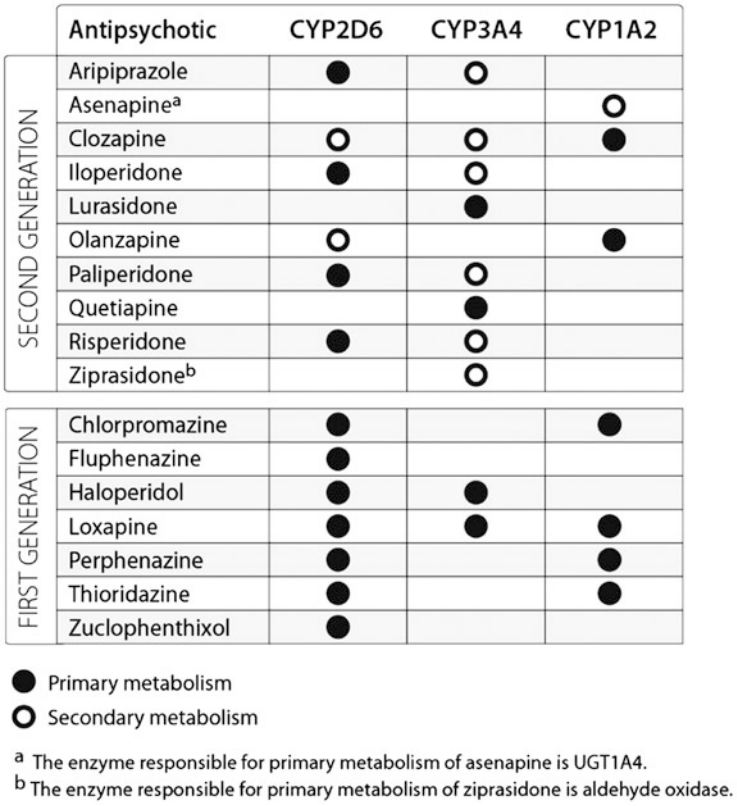

Fig. 6.1 Primary and secondary CYP450 enzymes responsible for metabolism of antipsychotics

patients) were usually described in detail in the labels of relevant drugs (e.g., aripiprazole and clozapine) [20].

Current commercial pharmacogenomic tests also regard *CYP* genes as a key factor to panel design, due to the great impact on AP response by altering drug plasma level. A number of reports have shown positive outcome of genetics-guided treatment using genotyping results of *CYP* genes [19, 21, 22]. On the other hand, several genes associated with AP treatment outcome have been reported to be candidate pharmacogenomic targets in clinical practice. SNP rs1799732 in *DRD2*, for example, has been found to be associated with the efficacy of risperidone [23]. MetS, a common adverse effect of APs which contributes to cardiovascular risks, has also been reported to be related to several genes. In particular, *HTR2C* and *MC4R* have been demonstrated to be the most relevant risk factors of weight gain [18].

6.2.2.2 Treatment of Treatment-Resistant Schizophrenia

Treatment-resistant schizophrenia (TRS) is raised in approximately 33% of schizophrenia patients who remained non-sensitive to the standard APs treatments [24]. Some genetic studies have shown an association between TRS and neurodevelopment candidate genes, dopaminergic/serotonergic systems, and cytochrome families [25]. However, few of these studies had significant findings independently replicated. Clozapine, typically the only drug to treat TRS, often leads to adverse reactions including agranulocytosis, seizures, and cardiotoxicity [24]. Several genetic variants, such as the Ser9Gly (rs6280) polymorphism of the *DRD3* gene, 452His/Tyr (rs6314) of the *5-HT2A* gene, and the C825T variant (rs5443) of the G protein subunit beta 3 (*GNB3*) gene, were found an association with clozapine-response [26]. Agranulocytosis is the major cause of clozapine underutilization [27]. Polymorphisms in human leukocyte antigen (*HLA*) genes have been reported to be related to clozapine-induced agranulocytosis (CIA), including *HLA-B 158 T* (rs1093) and *HLA-DQB1 126Q* (rs1762) [28]. Neurological physiotherapy like transcranial magnetic stimulation or electroconvulsive therapy (ECT) may be effective for TRS patients who respond poorly to APs [29]. Although the previously identified biomarkers appeared to have the poor prediction of sensitivity in clinical treatment on CIA, and most early studies were performed only in patients of specific ethnic, further studies are necessary before concluded.

6.3 Pharmacogenomics of Depression

6.3.1 Pharmacogenetic Studies in Depression

Depression, the most prevalent psychiatric disorder, is a disabling mental illness affecting more than 350 million people globally [28]. Antidepressant drugs (ADs) are currently available for patients with depression, including both first-generation and second-generation ADs. For instance, the former generation includes tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs), and the latter generation includes SSRIs, noradrenaline reuptake inhibitors (NRIs), selective serotonin and noradrenaline reuptake inhibitors (SNRIs), noradrenaline/dopamine reuptake inhibitors (NDRIs), noradrenergic and selective serotonergic antidepressants (NaSSAs), and serotonin antagonist and reuptake inhibitors (SARIs).

Although ADs have been used widely in depression, their therapeutic effects need further improvement. Approximately 30–40% of depression patients were not responsive to the first ADs dosage, and 60–75% of patients failed to achieve complete remission [29]. It has been shown that the benefit of the drugs only emerged after chronic treatment, which indicated that certain targets were still yet to be discovered. Clinical association studies have suggested that specific SNPs influenced the drug efficacy, and the combination of multiple genetic variants

contributes about 42% to the variation in antidepressant treatment response [30]. Most relevant studies have investigated genes involved in the metabolism encode receptors and transporters or other processes related to second-messenger systems.

6.3.1.1 Pharmacogenetic Studies of Pharmacokinetic Variation of Antidepressants

To date, *CYP450* and *ABCB1* genes have been identified to be associated with antidepressant treatment response in cumulative studies. The CYP enzymes are hepatic hemoproteins and responsible for the first phase of drug metabolism. The genes encoding these enzymes are highly polymorphic. The population of different ethnics has shown the difference in metabolizing capabilities. The altered metabolism rates may induce the alternation of drug plasma concentrations. Recent studies demonstrated that drug plasma levels were associated with metabolizer status in particular, such as PM and IM of *CYP2D6, CYP2C9,* and *CYP2C19* showed higher plasma levels and UM showed lower plasma levels after taking antidepressants [31, 32]. Polymorphisms in these genes may influence the metabolism of ADs, thus affecting drug efficacy and safety. Furthermore, *ABCB1* transporter gene, a member of the ATP-binding cassette superfamily of membrane transport proteins, may affect therapeutic efficacy through efflux transport in the blood–brain barrier (BBB) and limit the entry of lipophilic drugs into the brain. Previous studies have shown that the SNP rs2032582 (G2677T) in *ABCB1* was related to the expression and function of the protein, allowing greater penetration of ADs substrates through the BBB [33].

6.3.1.2 Pharmacogenetics of Antidepressant Pharmacodynamics

There are plenty of pharmacodynamics targets involved in the process of serotonergic neurotransmission (e.g., *SLC6A4, HTR2A*), glucocorticoid signaling (e.g., *FKBP5*), neuroprotection and neuroplasticity (e.g., *BDNF*), second-messenger cascades (e.g., *GNB3*), and metabolism of catecholamines (e.g., *COMT*). Recent pharmacogenetics studies on AD treatment response discovered that the monoaminergic candidate genes were associated with the serotoninergic system, which may underlie the mechanism of ADs. The *SLC6A4* gene encoding the serotonin transporter, which is the most studied genetic predictor of AD treatment response, has been found to be the principal site of action of TCAs, SSRIs, and SNRIs [34, 35]. *FKBP5* modulates the sensitivity of the glucocorticoid receptor and plays a major role in stress response and hypothalamic–pituitary–adrenal activity. Three polymorphisms in *FKBP5* gene (rs1360780, rs3800373, and rs4713916) were associated with the faster response to AD treatment and the increased recurrence of depressive episodes [30]. BDNF, a neurotrophin encoded by the *BDNF* gene involved in neuroplasticity, has been shown to have lower expression levels in

depressed patients and increased after the patients with antidepressive or electroconvulsive treatment. The most thoroughly investigated polymorphism in *BDNF* is rs6265 (Val66Met). Previous studies have shown that the rs6265 was associated with AD treatment response, and replicated by recent studies [36–38]. *GNB3* gene encodes for the beta polypeptide 3 of guanine nucleotide-binding protein (G protein) and functions as the cell signal transduction in response to a number of signals such as hormones and neurotransmitters. The T allele of *GNB3* rs5443 (C825T) polymorphism, a splice variant, altered the activity of its coding protein and associated with AD treatment response in the Asian population [39]. *COMT*, involved in the catabolic pathways of norepinephrine and dopamine, played a role associated with a remarkable effect on the activity of ADs, and the functional polymorphism rs4680 (Val158Met) was reported to influence the AD treatment response [35, 40].

Only a few genes have been validated to be associated with AD treatment response in pharmacogenetic studies, but the potential clinical application of these genetic signatures is yet to be explored. At present, GWAS and candidate gene studies are facing multiple challenges, which slowed down the translation process from biomarkers of antidepressant response found in scientific discoveries to the clinical applications. Therefore, paradigm changes such as the genome-wide theory may boost the development of pharmacogenetics for ADs and lead to discovery of more validated findings that can be utilized in clinical practice.

6.3.2 Treatment of Depression in Clinical Practice

6.3.2.1 Clinical Status of Antidepressant Treatment

With the substantial progress in the study of depression, various novel treatment approaches (e.g., psychotherapy, complementary and alternative medicine, exercise, and pharmacotherapy) have been developed and widely used in clinical practice. American College of Physicians has summarized and graded the comparative effectiveness and safety of nonpharmacologic treatments and second-generation ADs of depression [41]. National Institute for Health and Care Excellence has recommended the latest guideline of depression for children and young people [42]. However, we still lack solid evidence to make an explanation for individual differences in drug response of ADs [43]. More importantly, not many patients with depression were able to recover from the existing treatments due to a great number of huge challenges in the development of new therapy [44].

6.3.2.2 CYP Genes Related to Antidepressants Treatment Response

Most available ADs in current clinical practice are based on the metabolism process driven by the CYP family enzymes. In particular, *CYP2D6* and *CYP2C19* are the most studied genes for their significant relationship with the ADs treatment response.

The CYP2D6 is the main metabolic enzyme involved in the metabolism of several SSRIs (e.g., fluoxetine, paroxetine, and fluvoxamine) and most TCAs (e.g., amitriptyline, desipramine, and nortriptyline) [19]. Similarly, CYP2C19 has been found to be a major metabolic enzyme for citalopram, escitalopram and is of great importance to the metabolism of TCAs [45].

FDA highlights the dosage should be modified according to the activities of drug metabolism CYP enzymes in patients and puts it in drug labels of common ADs [20]. FDA also suggested that the frequent monitoring for using drug should be performed and the UM patients should avoid using amitriptyline. On the contrary, the dosage of amitriptyline and other TCAs should be halved for PM patients according to the drug labels. However, due to quite mixed influence of *CYP2D6* or *CYP2C19* metabolizer status on antidepressant response and side effects, the pharmacogenomic recommendations based on CYP genes in drug labels of different AD categories are not as explicit as those for TCAs (e.g., amitriptyline) [45]. Furthermore, FDA labels describe the instructions for co-administrated medications according to the effect of drug interactions on the metabolism of CYP enzymes. For example, when the *CYP2D6* inhibitor fluoxetine is used, attention should be paid to the co-administration of drugs metabolized by *CYP2D6* such as APs or other psychiatric medication. Nevertheless, as more evidence is generated in future studies, regulatory authorities were expected to approve and provide more recommendations from pharmacogenomic tests for clinical practice.

6.3.2.3 Pharmacogenomic Tests in Antidepressants Treatment

The pharmacogenomic test has greatly improved the ADs treatment and increased the remission rates of depression [46]. Previous studies found that the application of the pharmacogenomic tests in depressive disorders (especially treatment-resistant depression) was cost-effective via meta-analysis and pharmacoeconomic modeling [47, 48]. However, different tests have diverse panel design and annotation for variants [49]. As well-known target genes such as *CYP2D6*, *CYP2C19,* and *CYP3A4* have been widely used in commercial pharmacogenomic tests, recent studies have found that some other genes (e.g., *ABCB1*, *SLC6A4,* and *HTR2A*) also have significant associations with antidepressant responses and adverse events during treatment [19].

Neuroimaging studies of antidepressant response have found that the functional changes in the brain regions during treatment were associated with genetic variants [50]. The polymorphism of *SLC6A4* was associated with imaging outcomes in both depression patients and healthy volunteers who received ADs [50, 51]. These results have demonstrated that L-allele affected the amygdala reactivity, which was similar to the effect of SSRI treatment. Additionally, recent studies have found that *BDNF* and *FKBP5* may alter the cortical structures related to response [45]. Cumulative imaging-pharmacogenetics trials indicated that the phenotypes of specific brain regions may be associated with individual genetics and treatment response

[30, 45]. Thus, visualization and pharmacogenomic approaches may serve as the assistant tools for future antidepressant practice.

6.4 Pharmacogenomics of Other Psychiatric Disorders

6.4.1 Pharmacogenomics of Bipolar Disorder

Bipolar disorder (BD), a manic-depressive illness, is a common lifelong mental disorder with a prevalence of 0.8–2.4% all over the world [52, 53]. It is characterized by pathological disorder of emotion, including extreme elation, mania, and severe depression. There are three major categories of drugs for BD treatment, including mood stabilizer (e.g., lithium), antiepileptic and anticonvulsant drugs (e.g., valproate), and atypical APs (e.g., olanzapine and risperidone). Lithium has been used for more than 70 years and still be the most commonly prescribed medication for BD [52, 54]. However, only 60% of the BD patients have a satisfactory therapeutic response to lithium or lithium-relevant combinative therapies [52, 54]. Meanwhile, the therapeutic effects of other drugs remain heterogeneous, which reflected that patients were diversal in risk of BD [52, 54]. In addition, all the first-line drugs are at the risk of ADRs in patients [52].

Pharmacogenomics studies on BD have been carried out for nearly 20 years and have discovered more than 50 candidate genes associated with the relevant drug responses of BD treatment [52]. Most studies of lithium response have revealed multiple candidate genetic markers which were involved in the pathways of neurotransmitter system, inositol signaling, circadian signaling system, BDNF/TrkB signaling, neurotransmitters (e.g., adrenergic, noradrenergic, dopaminergic, GABAergic, glutamatergic, and serotonergic) systems, and other signaling processes (e.g., cyclic adenosine monophosphate response element binding protein). However, the findings of SNP markers associated with lithium response are currently limited in the research area and have not been translated for clinical diagnoses or prediction [55].

In addition to lithium, studies were also conducted on other first-line medical approaches for BD, including lamotrigine, valproate, and olanzapine/fluoxetine combination therapy. The polymorphisms of *DRD3*, *HRH1*, and *MC2R* were associated with the drug response of olanzapine/fluoxetine combination therapy [52, 53]. Similarly, *ANKK1*, *DRD2*, *DRD4*, *DBH*, *HRH1, MC2R*, and *NR3C1* have been shown to be significantly associated with treatment responses of lamotrigine [52, 53]. The polymorphisms of *XBP1*, *BDNF*, and *NTRK2* were associated with therapeutic responses of valproate [52, 53]. Most aforementioned genes were correlated to dopamine (D2, D3) receptors and histamine (H1) receptors [52, 53]. In addition, the different types of metabolic enzymes in BD patients were associated with the alternations in the drug response to ADs and APs [53]. For example, *CYP2D6* PM has been shown to have a higher risk of maniac episodes in BD after taking serotonin reuptake inhibitors, compared with all other three types of

drug users (UM, EM, and IM) [56]. Thus, the genotyping of CYP450 coding genes was recommended in clinical practice to optimize drug dosage for BD patients with ADs and APs treatment [53].

On the other hand, the use of anti-bipolar drugs may lead to ADRs. For example, lithium has been found to affect the renal function and reduce the ability of urine concentration, which may induce chronic interstitial nephropathy in patients [53]. Antiepileptic drugs may induce hypersensitivity, and atypical APs may lead to MetS, including weight gain and obesity [52]. In particular, recent studies reported a significant association between mitochondrial DNA polymerase gamma (*POLG*) Q1236H (rs2307441) variation and valproate induced liver injury [57]. This variation indicates a high risk of acute liver failure after valproate administration and leads to death in severe cases. FDA has warned this risk factor in valproate usage and highly recommended a gene test before receiving any relevant therapies containing this drug [53]. Recent studies have also shown that polymorphism in *GSK3B* gene may explain the differences in lithium-induced renal dysfunction across patients [52]. The weight gain and metabolic abnormalities induced by mood stabilizers and second-generation APs were associated with *GNB3, FTO, TBC1D1, MTHFR, HRH1, BDNF* (Met66 alleles), and gene promoter of serotonin transporter [52]. The polymorphism of *SLC6A4* has been found to be associated with mania induced by ADs [52]. However, no evidences showed that the relevant biological pathways of these identified genes were associated with the phenotypes [52].

6.4.2 Pharmacogenomics of Substance Abuse

Substance abuse, also called substance use disorders or drug use disorders, is a disease that affects the brain or behavior, and eventually leads to the loss of control to use of drugs in individual. Substance abuse leads to the impairment of primary life roles, suicidal psychology, neuropsychological deficits, and increased risk of infectious diseases, which could be significant economical burdens on both individuals and the society [53].

Opioid abuse is a common kind of substance abuse that has been found to be responsible for approximately 17,000 deaths per year in the USA [54]. Currently, the most effective pharmacotherapy has been suggested to be a combination of methadone, buprenorphine, and naltrexone [55]. However, this combination therapy has been found to be not effective for some patients, and genetics may play an important role in it [55]. The SNPs rs3745274 G>T of *CYP2D6*, rs1045642 G>A of *ABCB1* and rs4680 A>G of *COMT* were associated with the effective dosage of methadone in different ethnic populations. A recent study found that CGC/TTT heterozygotes of rs1128503-rs2032582-rs1045642 haplotype were significantly associated with the higher concentrations of dose-adjusted plasma concentrations of methadone in Malaysian patients with substance abuse [56]. The SNPs of *OPRM1* (rs3192723), *NTRK2* (rs2289658), and *UGT2B7* (rs7438135) also have been shown to highly related to effects of methadone. Additionally, a recent study discovered that the

genotype of rs678849 of *OPRD1* was associated with the treatment efficacy of buprenorphine in African Americans with opioid use disorders. These consistent results of two independent studies have suggested that the SNP rs678849 genotype may be an accurate pharmacogenetic marker for medication selection [55].

The CYP450 enzymes have been known to be able to metabolize a variety of chemical compounds. Genetic polymorphisms in *CYP450* genes alter the metabolic activity and transform clinical medications; therefore, polymorphisms in some *CYP450* genes are candidates for methadone-related death. Recently, 176 CYP haplotypes were identified based on the large combined data of the whole-genome and exome sequencing data from 56,945 unrelated individuals within five different ethnic cohorts [58]. Other genes in CYP450 family with less function alleles, specifically *CYP3A5* and *CYP2C9*, have been found to be associated with the increasing plasma concentration of methadone [59]. These results indicated that the enzymes involved in the metabolism of methadone, buprenorphine, and naltrexone in vivo may induce the adverse events. At last, the limited sample sizes and the lack of independent studies suggested that replicated pharmacogenomics studies were necessary to validate the discoveries before their application in clinical practice.

Another important kind of substance abuse is alcohol abuse or alcoholism. It is now usually treated by naltrexone, disulfiram, or acamprosate. As shown in PharmGKB (www.pharmgkb.org/disease/PA443309/clinicalAnnotation), genes *GATA4*, *DBH*, *OPRD1,* and *GRIN2B* have been regarded as important pharmacogenetic biomarkers of their drug efficacy (blunting of alcohol craving) in the treatment of alcohol abuse in mixed populations, and SNP rs1799971 GG+AG allele of *OPRM1* has been reported to be associated with increased severity of intoxication. Furthermore, the safety, tolerability, pharmacokinetic, pharmacodynamic, and behavioral effects of a novel ghrelin receptor inverse agonist, PF-5190457, have been suggested to be a potential pharmacological agent to treat alcohol use disorder in a recent phase 1b human laboratory study [60]. In sum, additional studies are expected to unveil increasing number of novel targets and markers that will facilitate the future drug developments for alcohol abuse patients.

6.5 Conclusion

Although almost 20 years passed since the first pharmacogenomic study, pharmacogenomics in psychiatric drugs is still in its infancy [61]. Until now, the most reliable biomarkers used in clinical practice are directly related with drug metabolism. Thirty-two markers on *CYP2D6* and 3 markers on *CYP2C19,* respectively, were written in the labels of 32 psychotropic medications approved by FDA [62]. However, the markers of neurotransmitter genes (e.g., *DRD*, *HTR*) that have been repeatedly reported in clinical studies were not as widely recommended for pharmacogenomics testing as CYP enzymes.

Although the predictive effects of current pharmacogenomic studies remain uncertain and most of them have not achieved convincing results, the benefits seem to outweigh the risks. In order to produce more reliable results, we need to overcome the limitations of sample size and the uncertainty of criteria defining phenotypes. Therefore, a study with larger sample size and sufficient feedback from the therapeutic approaches may generate more accurate results. The difference in phenotypic definitions, especially drug efficacy and ADRs, can lead to the heterogeneity in study. The consensus of evaluation criteria in drug effects, such as the application of standard scales, is essential. Moreover, the extrapolation of physiology results from pharmacological actions may be misleading. Due to the heterogeneity of drugs response, the search for commonalities in different psychotropic medications may also mislead. In psychiatric disorders, pharmacogenetics lags behind pathological studies, but it is gratifying that the former one is in rapid progress in recent years. In the past few years, the study of pharmacogenomics has gradually turned to genome association analysis and integration of multi-omics data [63]. It is believed that the pharmacogenomic studies on APs will be vigorously developed and be more effective in clinical practice in the near future.

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Chapter 7
Pharmacogenomics of Anti-Infective Agents

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Abstract Ever since we know that the dissimilarities of medications are influenced by the in vivo process of the body, including absorption, distribution, metabolization, and excretion, great attentions have been paid on pharmacogenomic issues, trying to figure out the underlying mechanisms of drug response deviations. Personalized regimens based on pharmacogenomic testing increased the efficacy and decreased the toxicity of certain drugs; therefore, correct understandings and utilizations of gene screening are of great benefits in rational medication usages. This chapter mainly introduced the pharmacogenetic information of some anti-infective agents, hoping to provide some tips for clinicians and other medical care providers.

Keywords Infectious diseases · Anti-infective agents · Genetic variations · Pharmacogenetics

7.1 Introduction

Infectious diseases are generally caused by pathogenic microorganisms. The invasions of microorganisms into human's body often cause different symptoms by reproducing and releasing toxins, some can be mild, but some could be lethal. For decades, infectious diseases have caused serious public health burden in China. As one of the most commonly prescribed drugs worldwide, anti-infective agents are severely tested due to the drug response variations, which include inadequate therapeutic efficacy and life-threatening adverse reactions.

The pharmacokinetic and pharmacodynamic properties of antimicrobials are quite distinguished from each other. Catalyzed by multiple enzymes, a plenty of antimicrobial agents may undergo extensive metabolisms which produce the active, inactive, or toxic metabolites, or be directly eliminated in parent forms. Differences in genetically encoded proteins result in different individual drug responses. Besides,

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_7

some severe adverse reactions are closely related to genetic variations, including drug hypersensitivity reactions.

Pharmacogenetics is the study of how polymorphic genes can alter drug disposition and therefore display different drug responses which may give us a better understanding of how genetic factors can be used to maximize the efficacy and minimize the incidence of adverse drug reactions. Therefore, further studies in pharmacogenetics are needed to help us to optimize the regimens of antimicrobial treatment precisely and wisely.

7.2 Antibacterial Agents

7.2.1 Amoxicillin–Clavulanate

As one of the most prescribed antibiotics worldwide, amoxicillin–clavulanate (AC) has presented the character of inducing drug-induced liver injury (DILI) in certain population, and accounts for 10–13% DILI-related hospitalizations. The idiosyncratic liver injury is thought to be linked to clavulanate. Evidences showed that the incidence of DILI induced by AC is much higher than amoxicillin alone. In 2011, the genome-wide association (GWA) study focusing on white European and US cases was conducted to investigate the linkages between genetic variants and AC-induced DILI, and it was confirmed that HLA class II SNP (rs9274407), which correlated with rs3135388 (a tag SNP of *HLA DRB1∗1501-DQB1∗0602*) was strongly associated with AC-DILI. Moreover, a SNP (rs2523822) in the region of the HLA-A locus, which was a tag for *A∗0201* allele, was also found to be related with the DILI susceptibility [1]. However, limited reports of AC DILI are reported in Chinese population, indicating that AC may be more tolerated in Chinese population.

7.3 Antifungal Agents

Antifungal agents are widely used because of the increasing number of immunocompromised, rapid growth of immunosuppressants usage, and the abuse of broad-spectrum antibiotics. Numerous studies revealed the correlations between genetic polymorphisms and the dispositions of azole agents, as it has been confirmed that genetic variation can result in different interindividual pharmacokinetic parameters as well as toxic reactions.

7.3.1 Voriconazole

Voriconazole is the most commonly used drug in clinical practice of invasive fungal diseases. It has been proved that voriconazole is extensively metabolized in liver by cytochrome P450, with only approximately 2% excreted in urine as unchanged form. Studies indicated that CYP2C19, 3A, and 2C9 contribute to around 75% of voriconazole's metabolism, and the rest 25% was processed by the flavin containing monooxygenase (FMO) [2].

It was reported that patients with *CYP2C19∗1/∗17* genotype were assigned to rapid metabolizers (RMs), *∗17/∗17* were classified as ultra-rapid metabolizers (UMs). Those with 1 copy of a *∗2* or a *∗3* allele were assigned to the intermediate metabolizer (IMs) phenotype, carriers of 2 copies were assigned to the poor metabolizer (PMs) phenotype, and patients without a *∗2*, *∗3*, or *∗17* allele were assigned to the normal metabolizer (NMs) phenotype [3]. According to a study that focused on the correlations between *CYP2C19* genotype and serum voriconazole exposure of 106 southwest Chinese Han patients, *CYP2C19∗2* and *CYP2C19∗3* allele presented in a relatively high frequency, about 45.3% and 41.5% of patients were categorized as NMs and IMs relatively, and 13.2% of patients were classified as PMs. In this study, the serum voriconazole concentration of PMs was almost 3 folds compared to NMs. Evidence showed that although the *CYP2C19∗17* frequency of Ethiopians/Swedes and Japanese was about 18% and 1.3%, respectively, the frequency was only 0.64% among Chinese male subjects, which indicated an absence of this mutation in Chinese, leading to an ultra-rapid metabolism of voriconazole [4].

The effects of *CYP3A5* on voriconazole metabolism are controversial. A study revealed that the *CYP3A5* variants were irrelevant to pharmacokinetic parameters of voriconazole [5]. Another study, in contrast, concluded that the frequency of certain genotype of *CYP3A4* was higher in patients with relatively lower serum voriconazole concentration. Therefore, the influences of *CPY3A5* variant on the pharmacokinetic of voriconazole need to be further verified. Besides, *CYP3A4* seems to have no functional impacts on voriconazole metabolism in Chinese [6].

According to the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline, the recommendations for voriconazole dosing can be chosen based on the patients' variants of *CYP2C19*. For those who were defined as NMs and IMs, standard dosing of voriconazole (intravenously: loading dose of 6 mg/kg IV every 12 h for two doses followed by 4 mg/kg IV every 12 h maintenance; orally: 200–300 mg every 12 h or 3–4 mg/kg every 12 h) is recommended; and for RMs and Ums, an alternative agent that is not affected by *CYP2C19* is recommended. PMs can also choose agents that are not impacted by the activity of CYP2C19; however, if voriconazole is the most appropriate agents, the dosage should be properly decreased and therapeutic drug monitoring should be performed in case of toxicity [7].

7.3.2 Itraconazole and Posaconazole

Although interacting with fungal CYP3A, itraconazole has limited influence on mammalian cytochrome P450 enzymes. The impacts of genetic variation on itraconazole have not been systematically conducted yet. However, an in vitro study demonstrated that itraconazole can inhibit enzymatic activity of CYP3A4 by fourfold, which indicated possible interindividual variations [8].

Unlike voriconazole, posaconazole is not metabolized by CYP enzymes, but undergo significant biotransformation catalyzed by uridine diphosphate (UDP)-glucuronosyltransferase (UGT) pathways [9]. It has been reported that *UGT1A4∗3* allele was related to the posaconazole plasma concentration, suggesting that variations in *UGT1A4∗3* may influence the clearance of posaconazole [10].

7.4 Antituberculosis Agents

Over 820,000 new cases of tuberculosis (TB) were reported in China in 2018, and the combination of multiple antitubercular agents is the cornerstone of TB treatment. Current first-line TB treatment regimen contains rifamycins (including rifampicin, rifabutin, and rifapentine), isoniazid, pyrazinamide, ethambutol, and streptomycin. Second-line TB agents include fluoroquinolones, capreomycin, amikacin, and kanamycin. Although current regimens are effective in most cases, there are still some intrasubject variants in drug responses and drug-related toxicities. Those variants are possibly resulted from the polymorphisms in drug-metabolism related gene. This part collected the information on drug-metabolic problems, hoping to provide insights into clinical use of antitubercular agents.

7.4.1 Isoniazid

Catalyzed by N-acetyl transferase 2 (NAT2), isoniazid undergoes deacetylation reaction in liver. It has been demonstrated that patients can be divided into rapid, intermediate, and slow acetylators. Previous studies have verified that the medical responses and toxicity of isoniazid are closely related to *NAT2* alleles [11, 12], and the activity of NAT2 is linked to the active alleles: *NAT2∗4* and *∗12*. According to the research, 9.3% of 172 Japanese subjects are slow acetylators (SAs), and 53.5% of them are rapid acetylators (RAs). Those without any active alleles are defined as SAs, and those are heterozygous are classified as intermediate acetylators (IAs). RAs have a greater possibility of treatment failure, whereas SAs have the chance of experiencing hepatotoxicity.

Also, study showed that it was worth trying to adjust the dosage of isoniazid accordding pharmacokinetic parameters. The study was carried out by giving SAs

reduced dosage of isoniazid than recommended one, and a lower dose was administered to those SAs. It turned out that genetic guided groups not only experienced lower incidence of isoniazid-related hepatotoxicity in SA groups, but also has a lower rate of treatment failure in RA groups [13]. A meta-analysis revealed that the percentage of PMs in 6285 Chinese subjects is approximately 21.1% [14]. And it has been concluded that all homozygous or heterozygous of *∗5*, *∗6,* and *∗7* are SAs in China, and the activity of the NAT2 is correlated with the gene-dose of the active alleles [15], which is proven to be the *∗4* and *∗12* alleles [16]. Therefore, the genotype of *NAT2* is a reliable predictor for the efficacy and isoniazid-induced hepatotoxicity of isoniazid.

7.4.2 Rifampicin

Rifampicin is mainly excreted unchanged through bile, and the polymorphisms of drug transporters may influence the pharmacokinetic of rifampicin greatly. Researchers found that the *SLCO1B1* rs4149032 polymorphism is linked with the low exposure of rifampicin in south Africans, and the frequencies of rs4149032 are 70% in Nigeria, 29% in Caucasians, and 56% in Asians, which may result in treatment failure [17]. However, *SLCO1B1* gene polymorphisms were found to have no impacts on the plasma concentration of rifampicin in south Indian subjects. Chinese researchers found that *SLCO1B1* rs2306283 was correlated with increased level of tacrolimus concentrations in renal transplant recipients, indicating that rs2306283 could possibly alter the treatment outcome of rifampicin [18].

In order to have a better understanding of impacts of genetic polymorphisms on antituberculosis agents associated hepatotoxicity, a population-based prospective case-control study was carried out in China. Among 927 enrolled subjects, researchers found that *SLCO1B1* and *UGT1A1*variants are closely associated with the incidence of antituberculosis drug-induced hepatotoxicity (ATDH). They found that patients carrying rs4149034 *G/A*, rs1564370 *G/C*, and rs2900478 *T/A* genotypes of *SLCO1B1* had a dramatically reduced incidence of having hepatotoxicity induced by antituberculosis agents, whereas rs2417957 *T/T*, rs4149063 *T/T* genotypes and haplotypes of *TGTG*, *TTTC*, *GTTC* were found to upgrade the risk. The *UGT1A1* polymorphisms also contributed the prevalence of ATDH; researchers found that the *UGT1A1* rs4148323 *A/A* genotype and haplotype *ATG* were related to the reduced risk of ATDH [19].

7.5 Antiviral Agents

HCV and/or HIV infection has become a serious public health issue in China and around the world. The incidence rate of HCV in mainland China is reported to be around 6‰, but it varies in different regions [20]. HCV infection often causes liver

cirrhosis, liver failure, and hepatocellular carcinoma if untreated. The dominant genotypes of HCV in mainland China are HCV-1 and 3, and over 95% of the subtypes of HCV are 1b, 2a, 3a, 3b, and 6a [21, 22]. Although direct-acting antiviral agents (DAAs) are available, the ribavirin/peginterferon alfa 2a (RP) regimen is still extensively used for the treatment of HCV infection, and the combination of RP regimen and DAAs remarkably improved the treatment efficacy.

There is no large-scale AIDS epidemic in China, but absolute number of HIV infected people is dramatically rising due to the huge population base of China. The regular application of antiretroviral therapy (ART) can achieve undetectable HIV RNA in patients' plasma. With the help of ART, the HIV-related morbidity and mortality are dramatically declined. However, poor patients' compliance of ART such as intolerance caused by undesirable drug responses and virologic failure due to insufficient exposures of ART constantly jeopardizes the control of AIDS epidemic. Among all the complex factors that affect the drug responses, pharmacogenetic analysis can be a good perspective of optimizing regimens and avoiding toxicities.

7.5.1 Peginterferon Alpha (PEG-IFN Alpha) Based Regimen

It has been unveiled that genetic variants in *interferon lambda 3* (*IFNL3*), also known as *interleukin-28* (*IL28B*) gene, are independently associated with the clearance of HCV, which is seen as a crucial predictor of the efficacy after RP regimen, certain genotypes of *IFNL3*. The frequencies of *C* allele at rs12979860 tend to be responsible for the dissimilarities on therapeutic outcomes and clearance of HCV [23, 24]. Study showed that HCV infected patients with rs1297860 *CC* genotype are associated with sustained virological response (SVR). Similar conclusion was observed in Chinese Han population with chronic hepatitis C. In this study, researchers found that *IFNL3* rs12979860 *CC* genotype linked to rapid SVR [25].

Giving to the correlations between *IFNL3* rs12979860 genotypes and the clinical outcomes, *IFNL3* genotyping is recommended by CPIC to predict the clinical outcomes. For patients with favorable response genotype, that is rs1297860 *CC* genotype, the chance of SVR is remarkably higher than patients with unfavorable response rs1297860 *CT* and rs1297860 *TT* genotypes [26].

7.5.2 Tenofovir

Several cases uncovered that tenofovir disoproxil fumarate (TDF)-treated patients experienced acute renal dysfunction and was possibly due to the TDF-associated cytotoxicity and DNA depletion [27–31]. It has been demonstrated that the elimination of TDF was related to the expression of *MRP4* (*ABCC4*) in vitro. Performed in *MRP4* knockout mice, researchers found that the kidney accumulation of TDF was obviously greater than the wild-type mice [32]. A study aimed to find the

correlations between TDF concentration and genetic variants revealed that patients with *ABCC4 4131 TG* or *GG* genotypes can increase TDF exposure by 30% than those with *ABCC4 TT* genotype [33].

A perspective study was conducted and they found a variant at position −24 of *ABCC2 (MPR2)*. Patients with *ABCC2 CC* genotypes exhibited a slower excretion for TDF, meaning that the TDF accumulated in epithelial tubular cells which possibly led to acute renal dysfunction [34]. The *CATC* haplotype at 1249, 3563, and 3972 in *ABCC2* was also reported to be associated with TDF-related renal dysfunction. Pushpakom et al. found that tenofovir is also a substrate of ABCC10, and the genetic polymorphisms can alter the plasma concentration of tenofovir [35]. TDF is also a substrate of human renal organic anion transporter 1(hOAT1), but limited evidences showed that hOAT1 is in concordance with TDF-induced kidney impairment.

7.5.3 Abacavir

Lot of studies have been carried out to unveil the connections between abacavir (ABC) and the hypersensitivity reaction (HSR) caused by human leukocyte antigen B (HLA-B). The HSR caused by ABC exposure is characterized by fever, rash, headache, and gastrointestinal discomfort. Symptoms of HSR may be mild at first, but can worsen rapidly as expoure to ABC increasing and can even lead to death. According to a retrospective study, the incidence of ABC-caused HSR was approximately 4.3% [36]. If suspicious cases are observed, the ABC must be immediately discontinued and the symptoms can be resolved. But re-exposure of ABC will lead to a more severe HSR.

The occurrence of HSR is closely linked to the polymorphisms of *HAL-B∗5701*. In a cohort study, researchers found that 78% of the patients with HSR carried *HLA-B∗5701* allele. The *HLA-DR7* and *HAL-DQ3* alleles were also found in patients with HSR [37]. Further studies about the linkage between HRS and *HLA-B∗5701* have been completed. Martin et al. renewed the criteria of diagnose HRS, with the help of this new criteria, researchers found that nearly 94.4% of the patients who experience HRS carried the *HLA-B∗5701* allele [38]. The frequencies of *HLA-B∗5701* are inconsistent among different ethics: the frequency of *HLA-B∗5701* is the highest in white and reached about 6.49%, and is the lowest in the black population (0.39%). The frequencies in American Indian and Asian are 3.48% and 3.61%, respectively. The study carried out in Hong Kong showed that among 792 Han-Chinese patients, only 4 (0.5%) of them carried *HLA-B∗5701*. And the frequencies in non-Chinese Asian and Caucasians were 1% and 3%, respectively. According to this conclusion, *HLA-B∗5701* screening may not be necessary in Han-Chinese population [39].

7.5.4 *Efavirenz and Nevirapine*

The non-nucleoside reverse transcriptase inhibitor efavirenz (EFV) has been used widely. With a narrow therapeutic window (1–4 μg/mL), the concentration of EFV varied inter-individually. The variations in concentration not only lead to different drug responses but also correlated with the adverse drug reactions. EFV is mainly catalyzed by CYP2B6 and the polymorphism of *CYP2B6* is the most important reason for the inter-patient variability in plasma concentrations [40].

516G>T has been extensively studied and found that the presence of T allele can result in lower levels of *CYP2B6* mRNA, and consequently lead to overexposure of EFV. According to researches, the concentrations of EFV in patients with *516 GT* and *TT* genotypes were higher than patients with *516 GG* genotype in Chinese population [41–43], indicating that a reduction of the dosage may be necessary to avert undesirable responses.

CYP 983T>C was also reported with the increased level of EFV concentration. Patients with one or two *C* alleles exhibited elevated EFV concentration compared to patients with *TT* genotypes. *983T>C* seemed to have a greater impact on EFV exposure and the frequency is less [44]. Moreover, Meng et al. reported that *171 +4335T>C*, *516G>T*, and *785A>G* were correlated with high levels of EFV concentrations, and suggested that they may be used for optimizing EFV dosage in Chinese [43]. Moreover, CPIC guideline points out that *CYP2B6* genotyping can be carried out in EFV-contained ART regimens to optimize dosage and avoid unexpected responses [40].

As with EFV, the plasma levels of nevirapine (NVP) are also linked to the polymorphisms of *516G>T*, *983T>C* and *15582C>T* which lead to increased plasma concentrations [45, 46]. HSR also occurred in 6–10% of the NVP-treated patients, which can be identified by rash, sever blistering skin reactions as well as hepatotoxicity. It has been unveiled that *CYP2B6 983T>C* is associated with the nevirapine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) and hepatotoxicity in Malawian and Ugandan HIV population which is probably due to the increased parent compound in circulating system [47]. Also, an association has been revealed between NVP-induced HSR and *HLA-DRB1∗0101* in patients with a higher $CD4^+$ T cell percentage [48], and then it has been reported that *HLA-B∗35*, *HLA-B∗58:01*, *HLA-C∗04, HLA-C∗08,* and *HLA-DRB1∗01* were relevant with HSR [49–53]. In a study focusing on genetic mechanism of the NVP-induced HSR in Chinese Han HIV-1 infected patients found that *HLA-Cw∗04* alleles were observed in HSR cases, and more *HLA-DRB1∗15* alleles were presented in NVP-tolerant group [54]. However, the intrinsic linkages between these alleles and NVP-induced HSR need to be further elucidated.

7.5.5 *Lopinavir/Ritonavir*

Lopinavir/ritonavir (LPV/RTV) has been successfully used for treated and untreated patients with HIV-1. Co-administered with ritonavir, lopinavir exhibits improved pharmacokinetic properties and it is tested to be well-tolerated and sustained suppression of the virus.

Both act as the substrates of CYP3A4, the pharmacokinetic and virologic outcome of LPV/RTV can be influenced by the polymorphisms of *CYP3A4*. Study showed that individuals with *CYP3A4∗22/∗22* had relatively lower clearances of lopinavir [55]. The study focused on the influence of genetic single nucleotide polymorphisms of *CPY3A5* on LPV/RTV monotreatment and found that a mean lower level of LPV/RTV was linked to the polymorphisms of *CYP3A5∗3* rs776746, *CYP3A5* rs28365088, *CYP3A5* rs15524, *CYP3A4* rs2687116, and *CYP3A4* nt20338, but these findings were not showing any significant differences [56]. Moreover, patients with *SLCO1B1 521T>C* are found to display a relatively higher LPV concentration, indicating that *521T>C* carrier may have a declined uptake of LPV [55, 57].

A prospective cohort study in children was conducted to try to clarify the effects of *CYP3A5*, *ABCB1*, and *SLCO1B1* genotypes on LPV/RTV pharmacokinetic properties and virologic outcomes showed that there is no statistically significant impacts of *CYP3A5*, *ABCB1*, and *SLCO1B1 388A>G* genotypes on LPV's pharmacokinetic and virologic outcomes, while *SLCO1B1 521T>C* genotypes were found to be associated with an increased LPV AUC [58].

7.6 Antimalarial Agents

Malaria, the most common infectious disease in the world, has become a serious public health issues worldwide and plenty of efforts have been made non-stop, trying to control and eliminate malaria globally. The treatments against malaria are recommended by WHO, and artemisinin-contained regimens are used according to the age and weight of the patients. The interindividual differences in pharmacokinetic parameters and undesirable drug responses suggest the genetically related factors can be crucial in treatment optimizing of antimalarial agents.

7.6.1 *Primaquine*

The finding of primaquine causing sever hemolytic anemia in glugose-6-phosphate dehydrogenase (G6PD) deficient population has become a major part in understanding the relationships between drug therapy and pharmacogenetics. According to the data, the frequency of this deficiency is high in malaria endemic areas, ranging from

5% to 20% in Asia and Africa. It has been demonstrated that the *G6PD 1388G>A* and 1376*G>T* are the most common variants in southern China. Patients with G6PD deficiency should avoid primaquine administration. However, no reports have shown correlations between hemolytic anemia and chloroquine monotherapy in G6PD deficient population. According to the research, the activation of primaquine is related to redox active metabolites via metabolism by CYP2D6. Further study clarified that primaquine showed no antimalarial activity in *CYP2D* knockout mice compared to those normal mice [59]; therefore, study focused on how CYP2D6 metabolizer status interfered primaquine efficacy and safety was conducted and found that *CYP2D6* IMs/PMs may be associated with longer gametocyte carriage [60].

7.7 Conclusion

The discoveries of how pharmacogenomics influence the efficiency and toxicity of antimicrobials have greatly benefited the treatments of infectious diseases. Dosing adjustment of EFV regimen based on *CYP2B6* polymorphisms, *HLA-B∗57:01* screening before ABC administration, predicting efficacy and toxicity of voriconazole based on *CYP2C19* variants, these applications in pharmacogenetics greatly improved the safety and effectiveness of antimicrobials. But further studies are needed to promote the understanding of how genetic factors affect the anti-infective treatment in China. As previously described, the applications of pharmacogenetics in treating infectious diseases are still limited due to the complexity of how genetic variants impact the pharmacokinetics and pharmacodynamics of antimicrobial, and we are only at the beginning of the precision medicines. More efforts must be made, and only in this way personalized medical care can be achieved eventually from every aspect.

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Chapter 8
Pharmacogenomics of Antithrombotic Drugs

Liyan Miao, Cheng Xie, Xiaoliang Ding, and Wenhao Qu

Abstract Antiplatelet drugs and anticoagulant drugs are the two predominant classes of antithrombotic drugs, which are mainly used to prevent and treat arterial thrombosis and venous thromboembolic diseases, respectively. How to modulate hemostasis while avoiding either thrombosis or hemorrhage is a very important issue in clinic. Although clinical factors remain of utmost importance in the decision-making process for the prescription of antithrombotic drugs, high throughput technologies are now opening up new routes to the identification of key genes that may allow more accurate personalization of antithrombotic therapy. In this chapter, we will use clopidogrel and warfarin as representatives of antiplatelet drugs and anticoagulant drugs to introduce the role of pharmacogenomics in their precise treatment.

Keywords Precision medicine · Pharmacogenomics · Antithrombotic drugs · Warfarin · Clopidogrel

8.1 Introduction

The human body maintains a delicate balance between bleeding and clotting, and optimal antithrombotic therapy aims to modulate hemostasis while avoiding either thrombosis or hemorrhage. Two predominant classes of oral antithrombotic drugs are antiplatelet drugs and anticoagulant drugs. Antiplatelet drugs are a cornerstone in the prevention of thrombus formation in patients with atherosclerosis like acute coronary syndrome (ACS) and thromboembolic stroke, while anticoagulants have proved to be more efficient at preventing venous thrombus formation and subsequent thromboembolic events, such as atrial fibrillation (AF), pulmonary embolism (PE), deep vein thrombosis (DVT), and mechanical heart valve replacement (MHVR).

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_8

Although clinical factors remain of utmost importance in the decision-making process for the prescription of antithrombotic drugs, high throughput technologies, such as genomics, are now opening up new routes to the identification of key genes that may allow more accurate personalization of antithrombotic therapy.

8.1.1 Mechanism of Antithrombotic Drugs

Platelet activation involves several mechanisms. Platelet adherence to subendothelial components, such as collagen, triggers a number of amplification pathways required for the formation of a stable thrombus. Soluble agonists, like thromboxane (TXA2) and adenosine diphosphate (ADP), are the main amplifiers of platelet activation and are the targets of the most prescribed antiplatelet drugs.

Vitamin K is an essential cofactor for the post-ribosomal synthesis of the vitamin K-dependent clotting factors. Warfarin acts by inhibiting the synthesis of vitamin K-dependent clotting factors, which include Factors II, VII, IX, and X, and the anticoagulant proteins C and S. Different to warfarin, novel oral anticoagulants (NOAC, e.g., rivaroxaban, dabigatran, apixaban, and edoxaban) specifically target a single coagulation factor. Dabigatran and its acyl glucuronides are competitive and direct thrombin inhibitors. Rivaroxaban, apixaban, and edoxaban are selective inhibitors of FXa and they do not require a cofactor (such as anti-thrombin III) for activity.

8.1.2 Pharmacogenomics of Antithrombotic Drugs

Inter-individual variability of drug response has been observed in clinical practice for many years. Polymorphisms affecting genes that encode disposition, metabolism, transportation, or targets of the drug can all potentially modify an individual's response to one therapy and thus explain its efficacy and safety profiles. Genotyping for gene selection or screening such as germline or somatic variants (polymorphisms, mutations), functional deficiencies with a genetic etiology, gene expression differences, chromosomal abnormalities, selected proteins that are used for treatment selection is used and is often based on genes categorized as known valid biomarkers by the US Food and Drug Administration (FDA) [1].

Because such inter-individual heterogeneity in antithrombotic drug response is less complex than the risk of developing cardiovascular diseases, the role of a single nucleotide polymorphism (SNP) affecting any step modulating the pharmacokinetics or pharmacodynamics of a drug could potentially be more relevant. Indeed, inter-individual variability in the pharmacodynamics and pharmacokinetics of several antithrombotic compounds have prompted numerous studies aimed at identifying the gene variants involved in their metabolism and targeting, such as warfarin (*VKORC1* and *CYP2C9*) and clopidogrel (*CYP2C19*). Recent advances in genomic

technology coupled with the development of new antithrombotic drugs have made this topic a dynamic and timely area of study.

8.2 Antiplatelet Agents

8.2.1 P2Y12 Receptor Antagonists

Clopidogrel and prasugrel are thienopyridine prodrugs that undergo hepatic biotransformation by multiple CYP enzymes to generate their pharmacologically active metabolite that irreversibly inhibits the ADP P2Y12 receptor. The conversion of clopidogrel to its active metabolite requires two sequential oxidative steps in the liver to form an intermediate metabolite (2-oxo-clopidogrel), and then an active metabolite (clopidogrel thiol H4). The majority (85%) of the drug is hydrolyzed to an inactive carboxylic acid derivative by carboxylesterase 1 (CES1), leaving only 15% of the absorbed dose for active metabolite formation, which occurs predominantly by CYP2C19. Figure 8.1 shows the representation of the candidate genes involved in the metabolism of clopidogrel and its primary mechanism of action [2]. Prasugrel is dependent on CYP3A4 and CYP2B6 metabolism to form its active metabolite. Ticagrelor is a reversible, pharmacologically active and noncompetitive P2Y12 inhibitor that is metabolized by CYP3A4 into a metabolite with equipotent antiplatelet effects.

The *CYP2C19* gene is highly polymorphic, with 37 known variant alleles up to September, 2019 [3]. The alleles most often studied in clopidogrel pharmacogenetics are *CYP2C19 ∗2* (*c.681G>A, rs4244285*), *∗3* (*c.636G>A, rs4986893*) and *CYP2C19∗17* (*c.-806C>T, rs12248560*). *CYP2C19∗1* is the normal function (wild type) allele, *CYP2C19∗2* and *CYP2C19∗3* are loss-of-function (LOF) alleles, and *CYP2C19∗17* is a gain-of-function allele. Polymorphisms in *CYP2C19* include LOF and increased function alleles, which combine to yield four CYP2C19 activity phenotypes: ultrarapid metabolizers (UMs; e.g., *∗1/∗17 or ∗17/∗17*), extensive metabolizers (EMs, *∗1/∗1*), intermediate metabolizers (IMs; e.g., *∗1/∗2* or *∗1/∗3*), and poor metabolizers (PMs, e.g., *∗2/∗2* or *∗2/∗3* or *∗3/∗3*). The *CYP2C19* allele frequencies vary considerably across ethnic groups. Approximately 30% of the white and black populations carry either 1 or 2 LOF alleles and are classified as IMs and PMs. However, the prevalence of IMs and PMs is markedly higher in Asian populations, with ≈60% of the population carrying a LOF allele. In addition, the prevalence of UMs in white and black populations is higher than in Asian populations, with about 30% and 4%, respectively [4].

It is well established that patients carrying LOF allele have a reduced capacity for clopidogrel bioactivation, impaired platelet inhibition, and an increased risk of major adverse cardiovascular events (MACE). In addition, carriers of *CYP2C19∗17* variant may have an increased risk of developing bleeding. Substantial evidence exists linking *CYP2C19* genotype with clinical outcomes among clopidogrel-treated ACS patients undergoing percutaneous coronary intervention (PCI) [5]. As a result,

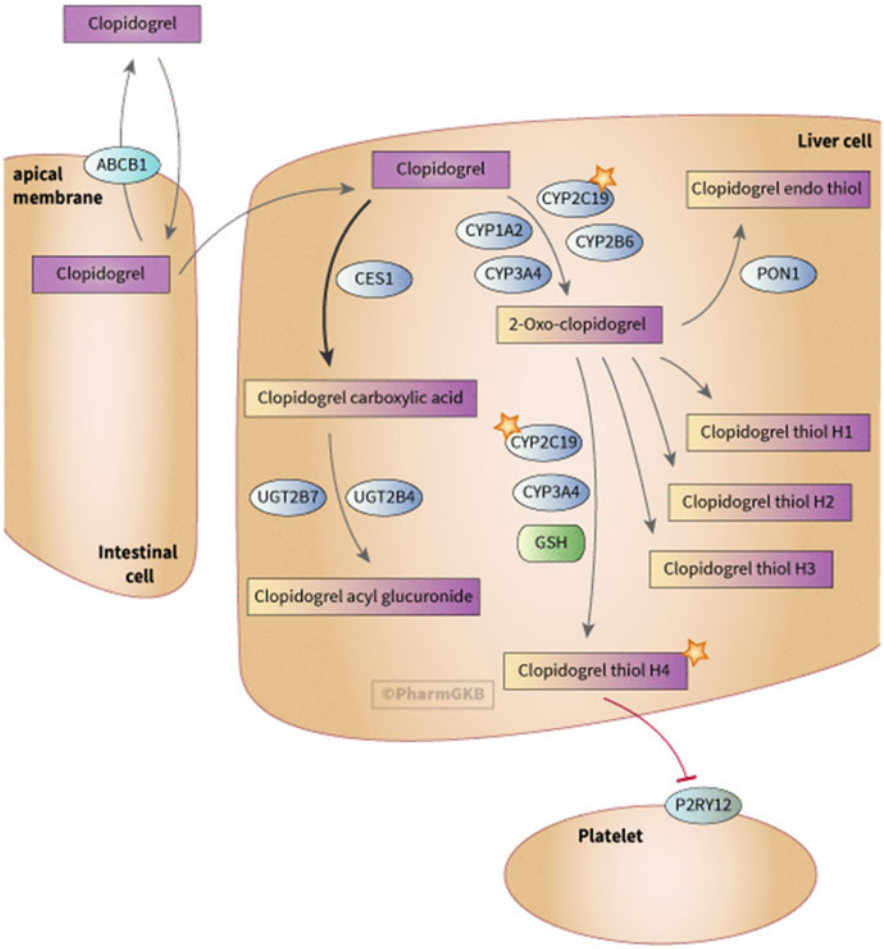

Fig. 8.1 Representation of the candidate genes involved in the metabolism of clopidogrel and its primary mechanism of action. Figure copyright PharmGKB. https://www.pharmgkb.org/pathway/PA154424674

the US FDA added a black box warning to clopidogrel advising against use in PMs in 2010. In contrast, *CYP2C19* genotype does not impact the clinical effectiveness of prasugrel and ticagrelor.

8.2.2 Aspirin

Aspirin is recommended as a first-line antiplatelet drug. Due to its widespread availability, low cost, lack of major adverse effects, and familiarity to both

physicians and patients, aspirin is the most commonly used antiplatelet agent worldwide. However, studies have revealed that in certain cases, the platelet function was inadequately inhibited, thereby leading to thrombotic events despite of the standard dose of aspirin, resulting in a range of individual response to aspirin therapy. This phenomenon has been termed aspirin resistance. Studies have observed that 5–65% of patients with ischemic stroke has aspirin resistance. Today, the underlying mechanism of aspirin resistance is still controversial, but it has been argued that genetic factors may be an important factor.

Aspirin inhibits platelet function through platelet cyclooxygenase-1 (COX-1) suppression, ultimately resulting in a decreased amount of TXA2. TXA2 is responsible for activation of platelet aggregation by binding to its specific receptor (TXA2 receptor, encoded by *TBXA2R*). Therefore, polymorphisms in this pathway may affect response to aspirin therapy. As the primary target of aspirin, COX-1 makes a logical enzyme for pharmacogenetic investigation. Polymorphisms in the COX1 gene, *PTGS1*, have been associated with variable response of platelet aggregation to aspirin. TXA2 receptor polymorphisms have also been associated with variation in aspirin response. The *TBXA2R CC (rs1131882)* genotype was found more frequently in the aspirin resistant group (81.8% vs 62.4%) than in the sensitive group and was identified as a risk factor for aspirin resistance (odds ratio = 2.712, 95% CI: 1.080–6.810) with a higher level of *AA*-induced platelet aggregation [6]. Fujiwara showed that aspirin was less effective for *924T* homozygote of a TXA2 receptor *and 924T>C (rs4523),* suggesting that *924T* allele is involved in aspirin resistance. However, there is some discordance in the literature, and others have not found a convincing association of variants and aspirin response [7].

8.2.3 Clinical Implications and Emergence of New Prospective Studies

With respect to professional statements, 2016 the American College of Cardiology/American Heart Association (ACC/AHA) guideline Focused Update on Duration Antiplatelet Therapy in Patients With Coronary Artery Disease [8] and 2017 the European Society of Cardiology (ESC) guideline Focused Update on Dual Antiplatelet Therapy [9] do not make a recommendation regarding *CYP2C19* genotyping, due to that no randomized controlled trial (RCT) has demonstrated that routine genetic testing to guide P2Y12 inhibitor therapy improves outcome. Clinical Pharmacogenetics Implementation Consortium (CPIC) [10] and Royal Dutch Pharmacists Association—Pharmacogenetics Working Group (DPWG) [11] have evaluated therapeutic recommendations for clopidogrel based on *CYP2C19* genotype. The therapeutic options are listed as shown in Table 8.1. As described in CPIC Guideline for clopidogrel and *CYP2C19*: 2013 update, standard dosing of clopidogrel is warranted among ACS/PCI patients who are CYP2C19 EMs or UMs. If genotyping identifies a patient as a CYP2C19 PM or IM, literatures support the use

Table 8.1 Antiplatelet therapy recommendations based on *CYP2C19* genotype from CPIC and DWPG guidelines

Genotype	CPIC 2013	DWPG 2018
CYP2C19 PMs	ACS patients managed with PCI (ACS/PCI): Alternative antiplatelet therapy (if no contraindication), e.g., prasugrel, ticagrelor	Percutaneous coronary intervention, stroke or TIA: Avoid clopidogrel. Prasugrel, ticagrelor, and acetylsalicylic acid/dipyridamole are not metabolized by CYP2C19 (or to a lesser extent) Other indications: Determine the level of inhibition of platelet aggregation by clopidogrel. Consider an alternative in poor responders. Prasugrel and ticagrelor are not metabolized by CYP2C19 (or to a lesser extent)
CYP2C19 IMs	ACS patients managed with PCI (ACS/PCI): Alternative antiplatelet therapy (if no contraindication), e.g., prasugrel, ticagrelor	Percutaneous coronary intervention, stroke or TIA: Choose an alternative or double the dose to 150 mg/day (600 mg loading dose). Prasugrel, ticagrelor, and acetylsalicylic acid/dipyridamole are not metabolized by CYP2C19 (or to a lesser extent) Other indications: No action required
CYP2C19 UMs and EMs	Label-recommended dosage and administration	No action is required

of an alternative antiplatelet agent (e.g., prasugrel or ticagrelor) when it is not contraindicated. In addition, clinical factors must be considered based on risk and benefit to most effectively individual therapy among clopidogrel-treated IMs. Another guideline from DPWG published in 2018, in patients who are undergoing PCI, stroke, or transient ischemic attack (TIA), they recommend avoiding clopidogrel use in CYP2C19 PMs, and choosing an alternative drug or doubling the dose to 150 mg/day (600 mg loading dose) in CYP2C19 IMs. No action is required for CYP2C19 ultrarapid metabolizers. It is important to note that the CPIC guidelines are designed to help clinicians understand how available genetic test results can be used to optimize drug therapy rather than to address whether routine genetic testing should be performed. Thus, it can be seen that routine genetic testing has not been recommended in current clinical practice guidelines because of lack of prospective evidence.

Several observational trials conducted in China, USA, and Europe have found *CYP2C19* genotype-guided antiplatelet therapy was superior to conventional therapy in patients undergoing PCI. The emergence of new evidence from prospective clinical trials of *CYP2C19* genotype-guided antiplatelet therapy has offered new insight and advanced the field to a critical inflection point.

In 2013, the Individual Applications of Clopidogrel after Percutaneous Coronary Intervention (IAC-PCI) study [12] was a prospective, randomized, single-center controlled study performed in 600 Chinese patients receiving PCI for coronary

artery disease. In the genotype-guided group, patients with IMs received 600 mg loading dose and 150 mg maintenance dose, and PMs received either high dose clopidogrel plus cilostazol. The incidence of MACE at 6 months was 2.6% in the personalized group but 9.0% in the conventional group, with no difference in bleeding risk. This is the first study to perform personalized antiplatelet therapy according to *CYP2C19* genotype in Chinese population. Recently published multicenter RCTs that examined clinical outcomes, Pharmacogenetics of Clopidogrel in Acute Coronary Syndromes (PHARMCLO) trial, POPular Genetics, demonstrated similar results.

The PHARMCLO study [13] was conducted across 12 centers in Italy, and 888 patients with ACS were randomly assigned to standard of care or genotype-guided arm, which used a treatment algorithm that considered *CYP2C19∗2*, *CYP2C19∗17*, and *ABCB1 (c.3435C>T, rs1045642)* genotyping results but left ultimate therapy choice to provider discretion. At 12 months follow-up, the primary endpoint (cardiovascular death, myocardial infarction, stroke or major bleeding) occurred in 71 patients (15.9%) in the genotype-guided arm and in 114 (25.9%) in the standard of care arm (HR = 0.58; 95% CI: 0.43–0.78; $p < 0.001$). The genotype-guided arm had a lower risk of ischemic events compared with the standard of care arm (13.0 vs 21.4%; $p < 0.0001$), with no statistically significant difference in major bleeding tween groups (4.2 vs 6.8%; $p = 0.1$). Although, the study was prematurely stopped after enrolling 888 of the target 3612 patients due to the lack of certification for the genotyping platform used in the study, it confirmed that a personalized approach to select antiplatelet therapy (clopidogrel, prasugrel, ticagrelor) on the basis of a patient's genetic and clinical characteristics leads to better clinical outcomes in comparison with the standard of care, which bases the selection on clinical characteristics alone.

A largest multicenter RCT performed at 10 European sites (8 in the Netherlands, 1 in Belgium, and 1 in Italy) has been published up to date, namely, POPular Genetics study [14]. 2488 patients undergoing primary PCI with stent implantation were enrolled and randomized to either a P2Y12 inhibitor on the basis of early *CYP2C19* genetic testing (genotype-guided group) or standard treatment with either ticagrelor or prasugrel (standard-treatment group) for 12 months. In the genotype-guided group, carriers of *CYP2C19* LOF alleles received ticagrelor or prasugrel, and noncarriers received clopidogrel. At 12 months, the primary combined outcome occurred in 63 patients (5.1%) in the genotype-guided group and in 73 patients (5.9%) in the standard-guided group. The genotype-guided group had a lower risk of bleeding compared with the standard-treatment group (9.8 vs 12.5%; HR = 0.78; 95% CI: 0.61–0.98; $p = 0.04$). The lower risk in genotype-guided group was not found because of usage of ticagrelor among over 90% patients in standard-treatment group.

Another ongoing large prospective clinical trials are expected to further contribute to our understanding, Tailored Antiplatelet Initiation to Lessen Outcomes due to Decreased Clopidogrel Response After Percutaneous Coronary Intervention (TAILOR-PCI) which will be the largest RCT of genotype-guided antiplatelet therapy to date [15]. TAILOR-PCI is an international trial with sites based in the

USA, Canada, Mexico, and Korea comparing point-of-care genotype-guided antiplatelet therapy (ticagrelor in IM/PMs and clopidogrel in non-IM/PMs) with routine care to determine whether identifying *CYP2C19* LOF allele patients prospectively and prescribing alternative antiplatelet therapy is beneficial. Emerging evidence for the clinical benefit of using *CYP2C19* genetic testing to guide antiplatelet therapy selection has important implications for clinical workflows in PCI patients.

8.3 Anticoagulants

8.3.1 Warfarin

As we know, warfarin is the most classic drug in pharmacogenomics research. It exists as racemic mixtures of S- and R-enantiomers, with the S-enantiomer being up to five times more potent than the R-enantiomer. The S-enantiomer of warfarin is mainly metabolized to 7-hydroxywarfarin by CYP2C9, a polymorphic enzyme. The variant alleles, *CYP2C9∗2 (C430T, rs1799853)* and *CYP2C9∗3 (A1075C, rs1057910)*, result in decreased in vitro CYP2C9 enzymatic 7-hydroxylation of S-warfarin. On the other hand, warfarin reduces the regeneration of vitamin K from vitamin K epoxide in the vitamin K cycle through inhibition of VKOR, a multiprotein enzyme complex. Certain single nucleotide polymorphisms in the *VKORC1* gene (e.g., *–1639G>A, rs9923231*) have been associated with variable warfarin dose requirements. *VKORC1* and *CYP2C9* gene variants generally explain the largest proportion of known variability in warfarin dose requirements. Figure 8.2

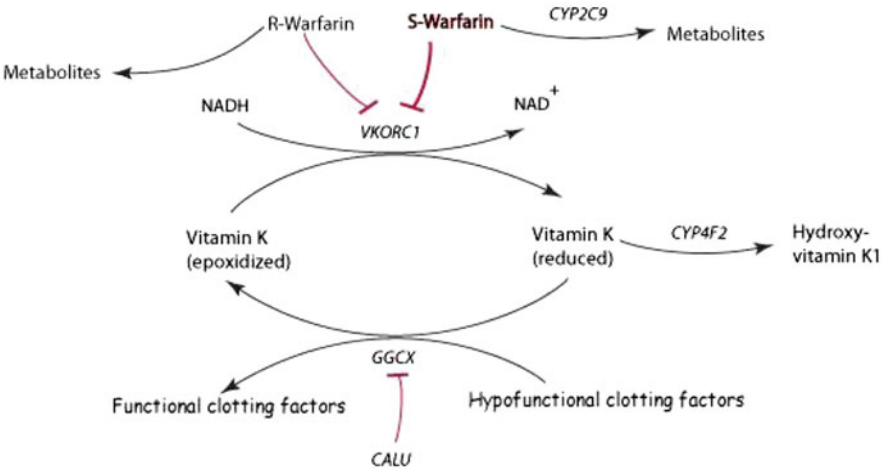

Fig. 8.2 Representation of the candidate genes involved in the metabolism of warfarin and its primary mechanism of action

shows the representation of the candidate genes involved in the metabolism of warfarin and its primary mechanism of action [16].

8.3.1.1 VKORC1

VKORC1 is the target site for warfarin. Warfarin exert its anticoagulant effects by inhibiting the VKOR, preventing VKOR from converting vitamin K epoxide to reduced vitamin K. Common *VKORC1* polymorphisms occur in the gene's regulatory region rather than coding regions that house the warfarin resistance mutations. Seven common *VKORC1* variants were initially associated with warfarin dose requirements in Caucasians, which comprised two major haplotypes, commonly designated as *VKORC1* haplotypes A and B [17]. Haplotype A is associated with lower mRNA expression and warfarin maintenance dose compared to haplotype B. Of the SNPs comprising *VKORC1* haplotypes A and B, only the *-1639G>A* and possibly the *1173C>T (rs9934438)* SNP appear to be functional. The *-1639A* and *-1173T* SNPs are in near complete linkage disequilibrium in individuals of European, Asian, and African descent. Thus, only one of these SNPs needs to be considered for warfarin dosing decisions. There is ethnic variation in the frequency of the *-1639A* variant. The frequency of the A allele is highest in Asians (~90%) and lowest in persons of African descent (10%), with an intermittent frequency in populations of European descent (~40%) [18]. As a result, the average dose of warfarin required in East Asian patients is significantly lower than that required in Europeans [19].

8.3.1.2 CYP2C9

The *CYP2C9* gene encodes the CYP2C9 enzyme, which is principally responsible for the metabolism of the pharmacologically more effective S-enantiomer of warfarin. The *CYP2C9∗2* and *CYP2C9∗3* alleles are the most extensively studied. *CYP2C9∗2* isoform leads to replacement of arginine at amino acid residue 144 by cysteine. This mutation reduces the catalytic activity of the enzyme to about 12% of the wild-type enzyme. *CYP2C9∗3* results in a substitution of leucine for isoleucine at amino acid position 359, which results in a reduction of catalytic activity to about 5% of wild type. The frequency of *CYP2C9∗2* in Caucasians is 13%, but in Asians is very rare or not detected in certain populations, such as Taiwanese [20] and Chinese [21], while the frequencies of *CYP2C9∗3* in Caucasians and Asians are 7% and 3% [22], respectively. The clearance of S-warfarin is reduced approximately 40% with the *CYP2C9∗1/∗2* genotype, up to 75% with the *∗1/∗3* genotype, and up to 90% with the *∗3/∗3* genotype [23]. Accordingly, individuals with the *CYP2C9∗1/∗2*, *∗1/∗3* or *∗3/∗3* genotypes required dose reductions of 30%, 47%, and 80%, respectively, compared to those with the *CYP2C9∗1/∗1* genotype [24].

8.3.1.3 CYP4F2

In contrast to the *CYP2C9* genotype, the *CYP4F2* genotype accounts for only 1% to 3% of the overall variability. The CYP4F2 enzyme is responsible for metabolizing vitamin K1 to hydroxyvitamin K1. This process results in less vitamin K1 being available for reduction to vitamin KH2, which is necessary for clotting factor activation. Thus, increased CYP4F2 activity leads to reduced clotting factor activation. Studies have demonstrated the association between the *CYP4F2* genotypes and dose requirements in Caucasians and Asians [25]. This finding could reflect ethnic differences in *CYP4F2* allele and genotype distribution and the minor contribution of *CYP4F2*, as well as the modulating effects of other more important dose requirement variables such as *CYP2C9* and *VKORC1*.

8.3.1.4 Others

Other genes, including calumenin and gamma glutamyl carboxylase (GGCX), produce lesser effects on warfarin pharmacodynamics and provide minor contributions to the variability in warfarin dose requirements [26]. Calumenin inhibits gamma-carboxylation of vitamin K-dependent proteins, suggesting that it may influence warfarin dose requirements. GGCX enzyme plays an essential role in the biosynthesis of vitamin K-dependent clotting factors by carboxylating protein-bound glutamate residues. Besides, Perera et al. [27] identified a SNP upstream of *CYP2C18*, *rs12777823*, that is significantly associated with warfarin dose requirement in African Americans and is independent of previous associations with *VKORC1* and *CYP2C9*. Patients carrying this SNP have a significantly lower stable dose of warfarin than do those without this variant, and the addition of this SNP improved the International Warfarin Pharmacogenetics Consortium (IWPC) algorithm by 21%.

8.3.2 NOACs

Dabigatran is administered orally as the non-pharmacologically active prodrug dabigatran etexilate. After absorption, this is rapidly hydrolyzed to dabigatran in the intestine and liver by CES1 and CES2. Dabigatran metabolism does not seem to involve, inhibit, or induce CYP450 enzymes. Rivaroxaban, apixaban, and edoxaban are pharmacologically active directly. In vitro and in vivo studies have demonstrated that all the three inhibitors of factor Xa are substrates of CYP450, particularly CYP3A4/5 and CYP2J2, and of P-glycoprotein. No literature exists to date on the clinical importance of pharmacogenetics in factor Xa therapeutics.

8.3.3 Clinical Implications

Warfarin is highly prone to both pharmacokinetic (PK) and pharmacodynamic (PD) variability, thus its therapy is monitored using the prothrombin time clotting assay, commonly reported as the international normalized ratio (INR). Healthy, untreated individuals have an INR of 1.0 and the target INR for warfarin anticoagulation is typically 2.0–3.0. However, the variability between patients is so severe that a standard dose can lead to responses ranging from no clinical effect to double-digit INRs that place patients at high risk of potentially fatal hemorrhaging.

The major goal of warfarin pharmacogenomics is to improve the accuracy of warfarin dosing and, consequently, to reduce the risk for adverse reaction with warfarin therapy. A large part of the inter-individual variation in response to warfarin has been linked to genetic polymorphisms in *CYP2C9* and *VKORC1*. In August 2007, the U.S. FDA approved the addition of pharmacogenomic data to the warfarin labeling. The U.S. FDA has provided specific dosing recommendations for those with *CYP2C9* and *VKORC1* variants as of January 2010. The recommended ranges for maintenance doses for genetic variants are included in the Coumadin package insert and should be considered on dose initiation.

Algorithms incorporating a patient's genotype, demographic factors, and comedications have been developed in an attempt to improve the prediction of initial warfarin dosing, such as multiple linear regression (MLR), artificial neural network (ANN), regression tree (RT), boosted regression tree (BRT), support vector regression (SVR), multivariate adaptive regression spines (MARS), random forest regression (RFR), and lasso regression (LAR). Several studies [28, 29] have shown that the algorithm based on MLR still has similar or even better predictive performance for Chinese patients. Of the available warfarin dosing algorithms based on MLR, the two derived from the largest populations and most commonly cited are algorithm by Gage and colleagues [30], and the IWPC algorithm [31], which could explain 40% of the variability in warfarin dose among Caucasians and approximately 25% among Asians and African Americans.

As we know, ethnic difference is one of the critical factors affecting individual warfarin variability. Previous studies have shown that algorithms based on the local ethnicity have better predictive performance than those based on mixed or other races [32–34] and until now, there are nearly 20 warfarin dosing algorithms based on Chinese patients. Table 8.2 shows the summary of these algorithms [35–41].

As stated earlier, warfarin is highly prone to both PK and PD variability, some PK/PD model [42] and dose-response model [43] have been developed in recent years. These studies showed the performance of warfarin dose prediction by PK/PD or dose-response model based on the Bayesian principle may be better than other models.

Meta-analyses of warfarin pharmacogenetic studies have suggested that genotype-guided dosing of warfarin significantly increased the time in therapeutic range (TTR) compared with clinical-only algorithms [44]. However, no differences in either major bleeding or risk of thrombosis have been seen [45]. The CPIC

Table 8.2 Summary of algorithms

Algorithm	Indications (%)	Variables	Sample (*n*)	Target INR	R^2
Miao et al. [35]	AF (28.7), DVT/PE (6.2), MHVR (65.2)	Age, weight, *VKORC1*, *CYP2C9*	178	1.5–3.0	62.8
Huang et al. [36]	MHVR (68.0), AF (27.8), DVT (4.1)	Age, BSA, *VKORC1*, *CYP2C9*	266	1.8–3.0	54.1
Wei et al. [37]	AF (100.0)	Age, weight, *VKORC1*, *CYP2C9*, *CYP4F2*, previous thromboembolism, amiodarone, β-blocker	260	1.5–3.0	51.7
Tan et al. [38]	MHVR (100.0)	Age, BSA, *VKORC1*, *CYP2C9*, number of increasing INR drugs, smoking habit, stroke history, hypertension	321	1.7–3.0	56.4
Lou et al. [39]	MHVR (49.1), AF (25.4), PE (25.5)	Age, weight, height, *VKORC1*, *CYP2C9*, *CYP4F2*, amiodarone, digoxin	323	1.5–3.0	65.2
Li et al. [40]	MHVR (51.3), AF (36.46), PE (12.24)	Age, weight, height, *VKORC1*, *CYP2C9*, amiodarone	384	1.8–3.0	68.2
Pei et al. [41]	MHVR (100.0)	Age, BSA, *VKORC1*, *CYP2C9*, *CYP4F2*	247	2.0–3.0	58.3

BSA body surface area

published clinical practice recommendations in 2011 for warfarin dosing based on a known *VKORC1/CYP2C9* genotype [46], while organizations such as the American College of Chest Physicians (ACCP) do not recommend genotyping due to insufficient evidence of benefit [47]. Based on current evidence and guidelines, we should combine conventional INR monitoring and clinical judgment with genotyping.

For example, there was a 72-year-old Chinese female patient (height, 155 cm; weight, 48 kg) with hypertension and hyperlipidemia and was on the treatment with amlodipine 10 mg/day and atorvastatin 20 mg/day. Her liver function and renal function were normal, and she had no history of allergic reactions. During hospitalization, atrial fibrillation was detected on her electrocardiography (ECG) during a routine evaluation and confirmed by repeated ECGs. Her CHA2DS2-VASc score was 3 (female, >65 years and hypertension) and cardiovascular physician recommended warfarin to prevent thromboembolic stroke secondary to her atrial fibrillation. Before starting warfarin therapy, amiodarone and low molecular weight heparin (LMWH) was given and tests for *CYP2C9* and *VKORC1* genotypes were suggested for the patient. The results showed her *CYP2C9* and *VKORC1* genotypes were *∗1/∗1* (wild type) and *-1639AA* (wild type), respectively, and baseline INR was 1.05. Using www.warfarindosing.org [30] and the IWPC [31] algorithms' prediction, she was begun on 1.875 mg daily (16:00) of warfarin with a target INR of 2.0–3.0. Subsequently, her INR was tested every 3–4 days and after 1 week was only 1.73 (07:00).

In order to adjust her warfarin dosage accurately, we used www.nextdose.org [43] based on Bayesian feedback method. The patient's demographic characteristics, genotypes, and INR at the current dosage were entered in the above Web. On the basis of calculation result, her warfarin dose was added to 1.875 mg daily and 2.5 mg daily alternately. Three day after adjusted, her INR was up to 2.18 and LMWH was withdraw. The dosage was maintained until discharge and neither bleeding nor thromboembolic events were occurred, during which her INR fluctuated between 2.02 and 2.57.

By genotyping of target genes and using the appropriate models, we optimized the patient's pharmacotherapy and improved her clinical outcomes. Physician and pharmacists should be well versed with pharmacogenomics to realize their patients' precise therapy.

8.4 Conclusion

Management of anti-thrombosis treatment using pharmacogenetics is a prospective area. Clopidogrel and warfarin are good examples of clinical application of genetic information. The data from genome-wide association studies are available with clopidogrel and warfarin, supporting the candidate genes involved in drug pharmacokinetics and pharmacodynamics are the key predictors of clopidogrel and warfarin response. The majority of pharmacogenetics research has focused on these candidate genes. Over the past decade, emergence of lots of evidence in the field of anti-thrombosis therapy based on the genetic information advanced the filed to clinical utility. Individualized dosage of warfarin therapy on the basis of *CYP2C9* and *VKORC1* genotypes has been implemented in clinical practice. Although genetic testing in clopidogrel treatment has not been recommended in current guidelines globally, the recently published RCT comparing genotype-guided treatment with control treatment has confirmed the benefit of the genotype-guided treatment. Finally, with the development of precision medicine, other research fields containing transcriptomics, proteomics, metabonomics, and microbiomes should be integrated in the field of pharmcogenetics, to explore clinical biomarkers and elucidate the underlying mechanism comprehensively. For example, genetic factors in *CYP2C9* and *VKORC1* have only explained about 40% proportion variability of warfarin, gut microbiome composition, especially vitamin K-producing bacteria, may differ each other or be disrupted by drug interaction and diet, leading to alteration in coagulation status. The emergence of new field may be translated to warfarin individual dosing in clinical practice.

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Chapter 9
Pharmacogenomics in Therapeutic Drug Monitoring

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Abstract Therapeutic drug monitoring (TDM) is useful in individualized therapy of immunosuppressants, antibiotics, and drugs used for anti-epileptic, anti-tumor, antiasthma. The drug concentration in blood or other biological samples is used to reflect therapeutic efficacy and toxicity. TDM is also termed as clinical pharmacokinetic (PK) monitoring and the individualized PK parameters are used for individualized therapy. Genetic polymorphisms of drug-metabolizing enzyme, transporters, and targets have substantial effects on the PKs and pharmacodynamics (PD). Pharmacogenomics is also an important tool in personalized therapy. There is difference and relationship between TDM and pharmacogenomics in the time of monitoring, samples of monitoring, results interpretation and application. Population pharmacokinetics (PPK) is developed on the basis of classical PK and statistics, which describe the disposition of drugs in vivo, including the population mean value and individual variation. The influence of various factors including pharmaogenomics on the PK can be estimated. Population pharmacogenomic–pharmacokinetic models are established to determine the impact of genetic polymorphism on the PK parameters quantitatively. Through Bayesian assay, the individualized regimen could be designed before drug administration and adjusted with TDM results after drug administration. The models are widely studied in the immunosuppressants, anti-tumor drugs, and anti-epileptic drugs.

Keywords Therapeutic drug monitoring (TDM) · Pharmacokinetic (PK) · Pharmacodynamic (PD) · Genotyping · Population pharmacokinetic (PPK)

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_9

9.1 Role of TDM in Precision Medicine

9.1.1 Progression of Conventional TDM

With the progress in pharmacokinetic (PK) and pharmacodynamic (PD) research, the in vivo drug concentration and the disposition have been recognized to be closely correlated with efficacy and toxicity, and therefore the in vivo drug concentration can be used as a good surrogate marker of the therapeutic endpoint [1, 2]. Large numbers of clinical and pharmacological studies have demonstrated that the correlation between the drug effect and the drug concentration in blood is strong [3, 4]. On the other hand, efficacy may differ substantially between different individuals under the same drug dose. For instance, clinicians usually prescribe 300 mg phenytoin to control epileptic but it works only in about 28.5% epileptic patients, does not work effectively in about 60% patients, and produces toxic symptoms in the remaining 11.5% patients [5]. Further research has proven that the drug plasma concentration is better correlated with the PD of the drug than the drug dosage in many circumstances.

The research of TDM is measuring the drug concentration in the blood or other body fluids by using fast and sensitive techniques; studying the relationship of the drug concentration with the therapeutic efficacy and toxicity under the guidance of pharmacokinetic principles, based on which the drug administration protocol is further designed or adjusted. In short, TDM is combination of analytical chemistry and clinical PKs in biological samples. TDM is also recently termed as clinical PK monitoring (CPM) under some instance. TDM is an important component of hospital pharmacy, which is progressed rapidly. As early as in the end of the 1960s, hospital pharmacists in the USA began using microbiological, spectroscopic, and chromatographic methods to analyze the drug concentrations in the patient's body fluids. In the 1980s, the emergence of the effective concentration range concept and the practice of individualized drug delivery protocol design provided a new effective and rational method of drug administration for clinical practices. In addition, the innovation of analytical techniques and the use of sophisticated instruments have greatly facilitated the development of new TDM methods [6]. For instance, the application of immunoassays (i.e., fluorescence polarization immunoassay (FPIA), enzyme-multiplied immunoassay technique (EMIT)) and chromatographic technology (i.e., high performance liquid chromatography (HPLC), capillary electrophoresis (CE), and liquid chromatography-mass spectrometry (LC-MS/MS)). These assays not only make it possible to monitor the concentrations of parent drugs, but also drug metabolites, free drugs, and enantiomers. TDM can have a wider range of application in these fields.

9.1.2 Conventionally Monitored Drugs

Clinically, not all drugs need to be monitored under any situation. Firstly, TDM is based on the existence of a correlation between the drug concentration and pharmacological effect. In addition, the drug concentration in blood or other body fluids is an indirect index of the pharmacological efficacy, and therefore TDM is unnecessary when the drug shows an objective and simple indicator of efficacy. For instance, blood pressure (BP) itself is an objective and simple indicator, and therefore observation of the degree of BP reduction alone can help knowing the efficacy of the anti-hypertensive drug and whether the dose is appropriate or not in most cases. It is also true for hypoglycemic drugs and diuretics. Finally, some drugs have a wide therapeutic range of blood-drug concentrations, and therefore empirical drug administration under the guidance of the clinician is sufficient to guarantee a safe and effective therapeutic outcome without TDM.

9.1.2.1 The Characteristics of Drugs that Need to Be Monitored

1. Drugs that have a relatively narrow therapeutic range of effective blood-drug concentrations. Drugs in this category are mostly those with low therapeutic indexes such as cardiac glycosides whose effective dose is close to the toxic dose. It is necessary to design and adjust the drug administration protocol meticulously according to their PK characteristics and the patient's condition, and observe the clinical reaction closely.
2. Drugs that may produce remarkable inter-differences in blood-drug concentrations and PKs with the identical dosage, such as tricyclic antidepressants. Genetic polymorphism of drug-metabolizing enzymes of these drugs may produce more than 10-fold PK differences among individual patients, which may induce toxic reactions.
3. Drugs that possess non-linear PK properties, especially when these non-linear PK properties occur within or lower than the range of the effective drug blood concentration, such as sodium phenytoin, theophylline, and salicylic acid.
4. Drugs that are expelled mainly through hepatic metabolism (such as lidocaine and theophylline) or renal excretion (such as aminoglycoside antibiotics), especially in patients with liver/renal dysfunction or failure, and those with gastrointestinal dysfunction who use some of these drugs orally.
5. Drugs that produce non-compliance or resistance during long-term administration in some patients; drugs that induce (or inhibit) the activity of hepatic drug-metabolizing enzymes and, therefore, reduce (or increase) the drug efficacy; or drugs whose efficacy alters due to unknown reasons.
6. Drugs that are suspected as having induced toxicity in patients, especially in cases in which the toxic symptoms of the drug are similar to those observed in over dosage while they cannot be differentiated clearly in the clinical setting, such as arrhythmia in patients receiving procainamide treatment, knowing that over

dosage of the drug can also induce arrhythmia. Another example is sodium phenytoin toxicity, in that it is difficult to differentiate between convulsion induced by sodium phenytoin and epileptic seizures.

7. Drugs that may affect the therapeutic effect when used in combination with other drugs due to drug–drug interactions.

9.1.2.2 Drugs and Therapeutic Range of Coventionally Monitored

Although there are a variety of drugs currently available for clinical use, only about 20 of them need routine TDM according to the above criteria (Table 9.1). It should be noticed that with in-depth research and emergence of new drugs [7–10], the range of TDM application is expanding, such as new anti-epileptic drugs (lamotrigine, oxcarbazepine, and topiramate), new anti-coagulants (dabigatran and rivashaban), antibiotics (teicoplanin, linezolid and voriconazole), azathioprine, and targeted anti-neoplastic drug (imatinib and dashatinib).

9.1.3 Application of TDM in Chinese Patients

TDM has emerged in China for more than 30 years and its development has experienced the following three stages: (1) the stage of monitoring therapeutic drugs mainly by means of blood-drug concentration analysis, in this stage, different assays for the determination of blood concentration were developed; (2) the stage of clinical pharmacological research mainly in the fields of clinical PKs and PD, in this stage, TDM samples of various drugs were collected and clinical PK parameters were estimated; and (3) the stage of patient-orientated clinical pharmaceutical services, in this stage, the importance of the individualized therapeutic regimen was emphasized. There is evidence that TDM or CPM can reduce the death rate of patients, shorten the time of treatment and hospitalization, and reduce the occurrence of adverse reactions.

Routine TDM windows of therapeutic drugs have been established through studies on large patient population. With the development of immunosuppressive therapy in postoperative patients undergoing organ transplantation, TDM of cyclosporin A, tacrolimus, sirolimus, and mycophenolic acid has played an important role in improving the survival rate of patients. Immunosuppressive agents have become the most important first-class drugs indicated for TDM in terms of the number of samples monitored and the scope of TDM application, signifying the clinical significance of TDM. In addition, great numbers of studies measured the blood concentrations of carbamazepine, phenytoin sodium, diazepam, and theophylline and found that 88% patients who administered these drugs below the therapeutic window developed clinical symptoms; significantly more patients who administered these drugs above the therapeutic window developed toxic symptoms;

Table 9.1 Commonly used clinical drugs that need TDM and their ranges of therapeutic concentrations

Action classification	Drugs	Range of therapeutic concentrations
Cardiac glycosides	Digoxin	0.5~2.0 ng/mL
	Digitalis glycoside	13~25 ng/mL
Anti-arrhythmic drugs	Lidocaine	1.5~5.0 μg/mL
	Procainamide	4~10 μg/mL
	Quinidine	2~5 μg/mL
Anti-epileptic drugs	Sodium phenytoin	10~20 μg/mL
	Phenobarbital	15~40 μg/mL
	Sodium valproate	50~100 μg/mL
	Ethosuximide	40~100 μg/mL
	Carbamazepine	4~12 μg/mL
Tricyclic antidepressants	Amitriptyline	0.15~0.25 μg/mL
	Nortriptyline	0.05~0.15 μg/mL
	Imipramine	0.2~0.3 μg/mL
	Desipramine	0.15~0.3 μg/mL
Anti-manic drugs	Lithium salt	0.8~1.4 μg/mL
Anti-asthmatic drugs	Theophylline	10~20 μg/mL
Antibiotics	Gentamycin	$C_{0:}$ 0.5~2 μg/mL $C_{p:}$ 5~10 μg/mL
	Amikacin	$C_{0:}$1~4 μg/mL $C_{p:}$ 20~25 μg/mL
	Vancomycin	$C_{0:}$ 5~10 μg/mL $C_{p:}$ 30~40 μg/mL
Anti-tumor drugs	Methotrexate	24 h: <40 μmol/L 48 h: <0.5 μmol/L 72 h: <0.05 μmol/L
Immunosuppressants	Cyclosporin	$C_{0:}$ 3 months after surgery: 250~400 ng/mL 6 months after surgery: 200~250 ng/mL 12 months after surgery: 150~200 ng/mL $C_{2:}$ 3 months after surgery:1000~1200 ng/mL 6 months after surgery: 800~1000 ng/mL; 12 months after surgery: 700~800 ng/mL
	Tacrolimus	9~15 ng/mL
	Sirolimus	5~15 ng/mL
	Mycophenolic acid	AUC: 30~60 μg × h/mL

C_0: trough concentration, C_p: peak concentration

and the clinical symptoms were controlled effectively in 90% patients who administered these drugs within the therapeutic window.

TDM should be performed strictly according to the operating manual and interpreted by the evidence-based outcome. Some expert consensus guidelines on TDM have been published both in China and abroad, such as the expert consensus on therapeutic drug monitoring for children in China [11], the expert consensus on

therapeutic drug monitoring of vancomycin in China [12], the expert consensus on therapeutic drug monitoring of anti-epileptic drugs of the International League Against Epilepsy (ILAE) [13], and the guidelines of therapeutic drug monitoring of immunosuppressants of the International Society for Therapeutic Drug Monitoring and Clinical Toxicology [14].

9.2 Pharmacogenomics and TDM

9.2.1 Development and Clinical Application of Pharmacogenetics

With 13 years international efforts on Human Genome Project (HGP), the map of 3.0 billion base pair sequences of human genomes has been completed in 2000. It is found that the 23 pairs of chromosomes contain less than 30,000 genes, based on which the concept of pharmacogenomics is developed, which mainly deals with the relationship of various gene mutations with efficacy and safety, and explains why single nucleoside polymorphism is the genetic foundation of producing individual differences in drug metabolism and reaction at the molecular level. The development of pharmacogenomcs interpreted some abnormal PK and PD phenomena that cannot be explained only by monitoring the blood-drug concentration. Although pharamacogenetics and pharmacogenomics are literally different, their research contents are both concerned with the exploration of genetic factors leading to individual differences of drug reactions. With the advent of the concept of precision treatment in recent years, pharmacogenomic testing has been more widely applied.

The influence of genetic polymorphism on phase I and phase II drug metabolism enzymes, transporters, and receptors have been suggested by plenty of studies in various populations. Modern laboratory techniques are now available for the determination of genetic variants influencing drug efficacy, metabolism, and occurrence of adverse effects. According to a survey in 2017 in China [15], pharmacogenomics testing was carried out in half of the 187 large hospitals in China take part in of survey. More than 90% of these hospitals had conducted this work for no more than 5 years. The three most frequently detected items at present are clopidogrel-related gene detection in 74% hospitals, warfarin-related gene detection in 65.6% hospitals, and folic acid-related gene detection in 56.7% hospitals. However, the clinical significance of many genetic variants still need evaluations by thorough clinical trials.

9.2.2 The Relationship Between Pharmacogenomics and Conventional TDM

9.2.2.1 Overall Comparison [16–18]

In the conventional sense of TDM, it can be supposed that the blood-drug concentration reflects the concentration at the site of drug action and therefore the drug concentration can be used to bridge PKs and PD. In molecular level, the PK process is partially determined by drug-metabolizing enzymes and/or transporters, while PD is controlled by drug target protein. It can be inferred combination of pharmacogenetics and TDM is a step further as compared with conventional TDM. Through pharmacogenomic testing, the genetic polymorphism of drug-metabolizing enzymes, transporters, and target or receptor proteins can be evaluated, which not only reflect individual differences in TDM results, but also better explain individual differences in efficacy and adverse reactions of many drugs, and is more valuable in the individualized therapeutic regimen design or regulation.

9.2.2.2 Phenotyping and Genotyping

1. Phenotyping
   Phenotyping of drug-metabolizing enzymes is an indirectly way to analyze genetic polymorphism through the individual metabolic capacity. Phenotyping is carried out by administering a specific substrate of the drug-metabolizing enzyme (probe drug) to the subject. After a certain period of time, the plasma or urine is collected to detect the concentration of probe drug and its metabolite [19, 20]. The metabolic ratio (MR) was calculated through the ratio of the probe drug/metabolite. According to the antimode, the subjects are classified as poor metabilizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), and ultra-rapid metabolizer (UM). As long as the experimental conditions of phenotyping are well controlled (such as all the subjects are with normal liver/kidney function and no concomitant drug use), the result can intuitively reflect the rate of drug metabolism in the human. The results of the TDM include the most frequently parent drug, under some instance, the metabolite of the drug reflect the efficacy or toxicity closely. The concentration of parent drug or metabolite may be considered partly as the result of phenotyping.
2. Genotyping
   Genotyping directly analyzes gene variation of the subject and, therefore, can identify individuals with abnormal drug metabolism or receptor activities quickly and accurately with consistent results. The advantages of genotyping are as follows: directly detecting gene information of an individual; causing minimal damage as compared with phenotyping, because DNA can be extracted from a mucosal scraping smear, hair, and saliva without being affected by concomitant

drug administration or change of the hormonal level and pathological condition. Nevertheless, genotyping also has some limitations, such as the functional significance of many specific genotypes remains unclear at present time. On the other hand, simpler and higher throughput methods are required for routine gene analysis of large-scale clinical samples.

9.2.2.3 Comparison of Pharmacogenomics and TDM

The ultimate goal of pharmacogenetic monitoring is to achieve the goal of personalized therapy, i.e. decide on the most effective and safest dose for a particular patient prior to drug administration. Therefore, research on pharmacogenetics attempts to forecast the reaction of an individual to a particular drug prior to administration and reach the goal of "tailoring" in drug administration. As is the case with the conventional TDM, pharmacogenetic monitoring also needs the cooperation between the pharmacist and the clinical laboratory. For instance, the main work of the pharmacist in current conventional TDM is to explain the result of drug concentration provided by the clinical laboratory. In future, the laboratory will also play an important role in pharmacogenetic monitoring, forecasting the course of disease and adverse reactions, other than performing ordinary drug concentration analysis, and therefore the pharmacist will bear greater responsibility in explaining these new laboratory findings.

1. Timing of monitoring: Pharmacogenetic TDM could be a valuable tool to decrease the rising medical cost. The advance in genetic polymorphism analysis permits the genotyping of specific drug metabolism enzymes to estimate the metabolic capacity prior to drug administration so as to select appropriate drugs and doses, thus enhancing the efficacy of the initial medication and reducing the medical costs by reducing the frequency of clinical visits of the patient. Pharmacogenetic information can be applied for individualizing drug administration of the initial dosage and indentify patients that may not respond to particular drugs. Unlike conventional TDM which can only take interventions after drug administration, pharmacogenetic TDM can provide interventions prior to drug administration (Fig. 9.1). For instance, most antipsychotic drugs are partially metabolized through CYP2D6. Phenotype detection of patients who are using these drugs may result in a wrong conclusion of false PM and false EM due to drug inhibition or induction, whereas genotyping can predict the metabolic activity prior to drug administration, which can not only avoid errors of phenotyping but also provide prospective information for medical decision.
2. Sample monitoring: In conventional TDM, the drug concentration needs the blood sample (or saliva sample in some cases) to be collected in steady state. To ensure the accuracy and reliability of the TDM result, patient compliance is extremely important. In contrast, genotyping can not only use blood samples. Other samples including saliva, hair root, or mucosal scraping can also be used. It does not require a concentration nor patient compliance. Another unique

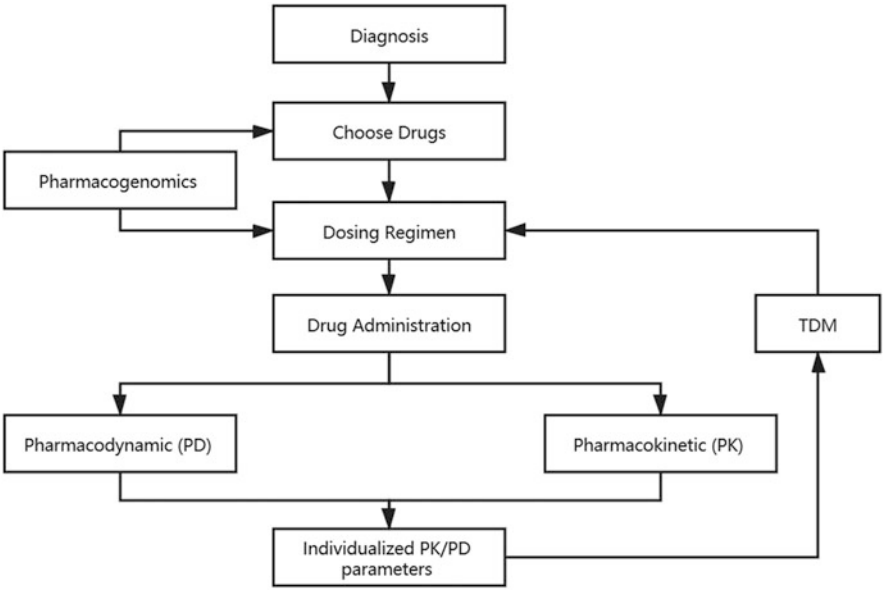

Fig. 9.1 Comparation of the application of TDM and pharmacogenomics in the duration of drug administration

difference is that conventional TDM can only provide predictive information of a single drug whose concentration can be determined, while TDM in combination with pharmacogenetics can provide predictive information of multiple drugs (such as multiple CYP2D6 substrates). Unlike conventional TDM which can only provide simple descriptive information, pharmacogenetics can obtain information why a particular patient needs a higher or lower drug dose, or information about the mechanism of other different candidate drugs.

3. Result monitoring: the genotype of a person remains unchanged over the lifetime. The drug concentration obtained by conventional TDM only reflects the PK characteristics of the patient at the given time point, owing to the fact that the effects of environmental and non-genetic factors on PK and PD may change with time. In addition, the pharmacogenetic information can improve compliance of the patient during drug administration. For instance, when the patient learns that he/she has been selected as the preferred target for gene detection of a particular drug, he/she will more comply with the therapeutic protocol, especially when the effect is achieved as expected. At the same time, the patient can avoid using expensive but ineffective drugs.
4. Ethical monitoring: In conventional TDM, the pharmacist and other medical workers may bear certain responsibility if the patient develops adverse reactions due to drug toxicity without detection of the drug concentration in advance. Pharmacogenetic TDM may also bring about additional ethical, legal, and social concerns. The pharmacist and other medical workers play an important role in

protecting the genetic information of the patient. Indeed, ethical issues in pharmacogenetic monitoring may not be so serious as those in gene analysis of certain diseases. Unlike some genetic diseases due to congenital metabolic defects, pharmacogenetic variations may not be harmful before drug administration. Identification of pharmacogenetic variations can avoid ineffective drug treatment and/or severe toxicity and adverse effects, which is beneficial to the individual lifetime.

5. Monitoring constraints: There are also shortcomings with pharmacogenetics based TDM. Clinical practices have demonstrated that many environmental, physiological, and pathological factors may affect diversities brought about by genetic factors. Individual differences in dosage and the range of target concentrations caused by these factors and other non-genetic factors are problems that conventional TDM still faces, which cannot be solved solely by genetic testing. In addition, the treatment target concentration has to be regulated from time to time with pharmacodynamic differences, in which case conventional TDM is the only means to achieve the goal of individualized treatment.

It is preferable to perform TDM by combining the conventional mode with pharmacogenetic TDM in selected patients. For instance, combination of conventional TDM and genotyping (or phenotyping) will make it easier to identify and correctly manage individual patient who are easy to present excessively high or low serum concentrations of antipsychotic drugs. Drug concentration monitoring is recommended for patients who are suspected with CYP2D6 defects and receiving the antipsychotic drug risperidone. At the same time, drug concentration monitoring is also useful in patients with CYP2D6 or CYP2C19 PM who are using selective serotonin reuptake inhibitors (SSRIs). Combination of conventional TDM with pharmacogenomics may provide more information on rational use of drugs. We can design a dosage regimen prospectively by using specific genetic information of the patient, hence the conventional TDM can be used to monitor whether the drug concentration is within the therapeutic range of the patient.

9.2.3 Clinical Application of Pharmacogenomics and TDM

9.2.3.1 Drug-Metabolizing Enzymes

Phase I Metabolizing Enzymes

1. CYP2D6 enzyme catalyzes metabolism of a large group of clinical therapeutic drugs, including many cardiovascular (i.e., codeine, propranolol, metoprolol, and propafenone) and central nervous system (CNS) drugs (perphenazine, haloperidol (HAL), zuclopenthixol, thioridazine, and risperidone). PM patients have higher blood concentration and higher risk of side effects. On the other hand, patients with multiple functional alleles may result in lower plasma concentrations than expected at conventional doses, and the treatment effect may be weak

or even absent. Some studies suggested that CYP2D6 phenotyping and genotyping can be used simultaneously to predict the concentration under steady stage (Css).

(a) Risperidone (RISP) is an atypical antipsychotic that potently blocks serotonin 5-HT2 and dopamine D2 receptors. RISP is converted to 9-hydroxyrisperidone (9-OH-RISP) through metabolism of CYP2D6.
In a study on 71 healthy subjects, the RISP and 9-OH-RISP levels and CYP2D6 genotype were determined. It was found AUC and $t_{1/2}$ of RISP and 9-OH-RISP correlated well with the number of CYP2D6 active allele. Hinrichs established a semi-quantitative gene dose model of CYP2D6, they found after RISP administration for 16 h, the MR (ratio of RISP and 9-OH-RISP) can be predicted by using the number of active CYP2D6 alleles: $\lg(\mathrm{MR}) = -0.51 \times N + 0.51$ [21].

RISP may be used in combined with SSRI to treat the negative side effects. SSRI is both the substrate and inhibitor of CYP2D6. A study on 11 patients with various CYP2D6 phenotypes (8 EMs and 3 PMs) administered with RISP and fluoxetine, showed RISP AUC in EMs before and after fluoxetine administration were 83.1 ± 46.8 and 345.1 ± 158.0 ng·h/mL. For PM patients, the level was 398.3 ± 33.2 and 514.0 ± 144.2 ng·h/mL. There are fourfolds and 1.3 folds increasing in AUC, respectively [22].

(b) Oosterhuis reported a 51 years old female patient, the dose of aripiprazole was increased from 15 to 30 mg per day. The symptoms of lethargy and memory loss were found after approximately 2 weeks therapy. After testing blood samples, the serum level of aripiprazole in this patient was 2990 ng/mL, approximately seven times the expected plasma concentration at the maximum dose of 30 mg/day. CYP2D6 of the patients were determined as ∗4/∗4 genotype; however, the CYP2D6 phenotype of this patients was transferred to PM. According the results, the aripiprazole was replaced by quetiapine [23].

2. CYP2C19 is another important phase I metabolizing enzyme. There are two main genetic polymorphisms which have significant impact on the metabolic activity. *CYP2C19∗2* cause the abnormal splicing error and *CYP2C19∗3* cause premature termination codon. Homozygous patients expressing "invalid" alleles are categorized as PMs and are highly sensitive to drugs such as diazepam, propranolol, antipyrine, and some proton pump inhibitors.
In particular, the antiplatelet drug clopidogrel transforms to an active component via CYP2C19. PM subjects have a higher risk of developing thrombosis due to reduced transformation of clopidogrel.

Voriconazole is a broad-spectrum triazole antifungal agent and the first-line drug for the treatment of invasive aspergillosis, which is often used for the clinical treatment of fatal fungal infections. It is metabolized via CYP2C19, CYP2C9, and CYP3A4, showing a great individual difference in blood-drug concentrations. The therapeutic window of voriconazole is relatively narrow, its PKs is non-linear, and the polymorphism of CYP2C19 gene is related to the individual

difference in PKs, all of which increase the risk of drug interactions and individual difference in blood-drug concentrations. TDM is highly recommended in some patients. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) suggests initiation of TDM 2–5 days after the use of voriconazole and dose adjustment until the concentration reaches the therapeutic range. On the other hand, it is difficult to reach the effective trough concentration in patients with ultra-fast metabolism of CYP2C9∗17, some researchers suggest that CYP2C19 gene analysis should be performed firstly to decide whether it is necessary to replace voriconazole with other antifungal agents in patients with aspergillus infection who need to reach a sufficient blood-drug concentration, especially in patients with aspergillus infection involving the CNS [24, 25].

Phase II Emtabolizing Enzymes

1. N-acetyltransferase2 (NAT2) is an important phase II enzyme, which catalyzes acetylation of aromatic amines and hydrazines. NAT2 activity exhibits remarkable polymorphism, which is mainly caused by NAT2 genotypes. There are seven single nucleotide polymorphisms (SNPs) in human NAT2 gene, consisting more than 27 different NAT2 haplotypes.
   Isoniazid (INH) is widely used in the treatment or prophylaxis of tuberculosis. Some studies indicated that there are great inter-individual differences of efficacy and toxicity of INH. Donald et al. [26] found ratio of C_{max} to the MIC of INH could be used to predict the early bactericidal activity of infections with Mycobacterium tuberculosis. Jayaram et al. [27] found cumulative antibacterial effect of INH related well to AUC and C_{max}. TDM of INH may be helpful for some patients. INH is mainly acetylated by NAT2 in human liver, and acetylisoniazid (AcINH) was major metabolite. Polymorphism of NAT2 activity was considered as the main reason of inter-individual difference of PK and drug response of INH.

   Kinzig-Schippers et al. studied various factors that influence the PKs of INH, and found *NAT2* genotypes accounted for 88% of variability in apparent INH Cl. The individual INH clearance could be estimated as Cl = 10 + 9 × (number of ∗4) [28]. More recent studies suggested that various *NAT2* SNPs have different influence on the metabolic activity of NAT2. Meisel et al. [29] found a quantitative relationship between various mutant patterns and the NAT2 activity using multiple linear regression methods. They concluded that MR of NAT2 can be represented as the equation: MR = 1.85–1.20 × (M282) − 1.28 × (M341).

   We established quantitative relationship between NAT2 mutant patterns and MR of 60 Chinese subjects, and verified the equation by the other 60 subjects. 80% of which were in ±20% range of the prediction error [29]. Besides, we also found INH PK parameters can be predicted from genotyping data of NAT2 in Chinese subjects. The PK parameters such as k, C_{max}, AUC, Cl of INH and C_{max}, AUC of AcINH can be calculated by NAT2 variant patterns. There was good correlation between observed and calculated data ($r^2 > 0.75$, $P < 0.0001$) except for C_{max} of INH ($r^2 = 0.32$, $P = 0.021$). The median prediction error for models from MR_{INH} was <±10% (range, −1.0–4.9%). The 95% confidence intervals for

prediction error ranged from −4.6%~8.9% of k to −11.6%~34.8% of C_{max} (Table 9.2) [30].

2. Purine drugs are commonly used for inflammatory bowel disease (IBD). Data show that about one-third of IBD patients may discontinue the use of the prescribed purine drugs because of the adverse reactions or resistance to these drugs. Observations of the individual differences in the therapeutic efficacy and adverse reactions show that this may be related to the activity of some key enzymes in active metabolites or purine metabolism.
   Azathioprine (AZA) is the precursor drug of 6-mercaptopurine (6-MP), which further metabolizes into active 6-thioguanine (6-TGN), 6-methylmercaptopurine (6-MMP), and other active metabolites in the body via metabolism. Bone marrow (BM) suppression induced by purine drugs is related to the hyper-concentration of 6-TGN and hypo-activity of TPMT. There are great individual differences in TPMT activity, which is caused by TPMT genetic polymorphism. Currently available studies have reported 31 mutation types (TPMT∗2–TPMT∗29) relative to reduced enzyme activity. TPMT∗2, TPMT∗3A, and TPMT∗3C account for about 60~95% of deficiency of enzyme activity. For homozygous mutant patients, the main metabolic routine of AZA is the production of 6-TGN rather than methylation, which may be more likely to induce severe BM suppression. The initial dose of AZA should be reduced to <10% of the recommended dose in such patients, or other drugs should be selected. For heterozygous patients, who have moderate TPMT activity, the dose should be reduced by 50%. For wild type patients, the recommended dose by weight can be initiated directly. Detection of the TPMT genotype prior to drug administration can predict or avoid severe and life-threatening BM suppression in patients with deficiency of enzyme activity. Rational detection of TPMT can reduce the risk of adverse drug reactions by 3~7%.

   However, about 70% patients with BM suppression have normal enzyme activity, which may be attributed to other purine-related enzymes, concomitant drug use, complicated infection, and immune-mediated drug response. For these patients, the dose of purine drugs needs to be increased under strict monitoring. Some clinical guidelines in recent years suggest detection of the 6-TGN and 6-MMP concentrations, which is reported to increase the clinical therapeutic efficacy by 15–30% in IBD patients. Concentration monitoring is strongly recommended in patients who are scheduled to increase the dosage. Studies have demonstrated that the therapeutic window is 230–260 pmol/8 × 10^8 RBC (Table 9.3). In conclusion, TPMT genotype detection in combination with TDM of important intracellular metabolites is of primary importance for rational use of purine drugs in IBD patients (Fig. 9.2) [31, 32].

Table 9.2 The equations for the determination of INH PK parameters

Equations	F	P	r2
$K(h^{-1}) = 0.61 - 0.24 \times (\text{M341}) - 0.20 \times (\text{M590}) - 0.18 \times (\text{M857})$	57.16	<0.0001	0.896
$t_{1/2}(h) = 1.04 + 1.86 \times (\text{M341}) + 0.99 \times (\text{M590}) + 0.83 \times (\text{M857})$	84.66	<0.0001	0.927
$C_{\max}$ (INH) ($\mu g\ mL^{-1}$) = 4.55 + 1.864 × (M590) + 1.69 × (M857)	4.07	0.021	0.370
AUC ($\mu g \cdot h \cdot mL^{-1}$) = 7.91 + 12.19 × (M341) + 5.38 × (M590) + 9.88 × (M857)	28.02	<0.0001	0.899
Cl ($L \cdot h^{-1}$) = 30.44 − 7.05 × (M341) − 11.26 × (M590) − 13.38 × (M857)	30.74	<0.0001	0.901

Table 9.3 TDM of purine metabolites at 8 weeks of drug administration in IBD patients

6-TGN[a]	6-MMP[b]	Explanation
Not detected	Not detected	Poor drug compliance
Low	Low	Poor drug compliance or insufficient dosage
Low	High	Main route of 6-MMP metabolism
High	Low	Therapeutic dosage or purine resistance if the therapeutic effect is not good
High	High	Excessive dosage or purine refractory type if the therapeutic effect is not good

[a]250 pmol/8 $\times$ 10^8 RBC
[b]5700 pmol/8 $\times$ 10^8 RBC

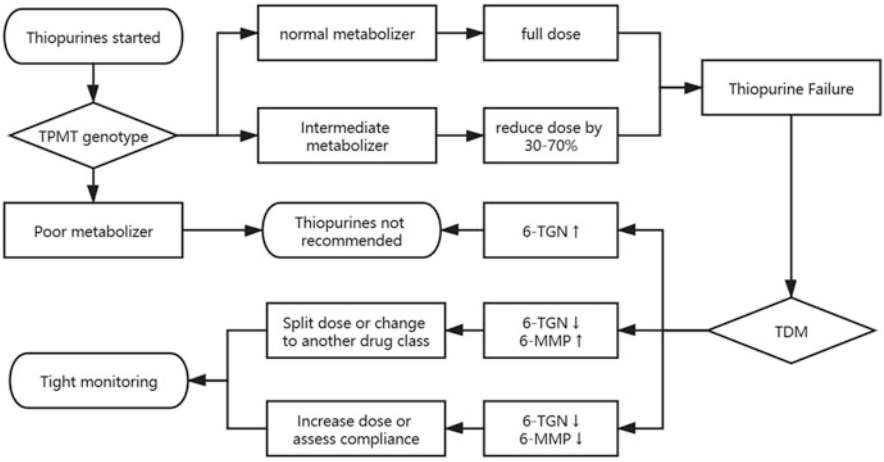

Fig. 9.2 Implementation of pharmacogenomic testing and TDM in use of thiopurines in inflammatory bowel disease

9.2.3.2 Transporters

1. SLC transporters
   The polymorphism and activity of transporter genes have significant impact on the PK process of some important drugs, which further affects the drug efficacy and adverse reactions. A previous study [33] observed the MPA PKs in 42 healthy volunteers with different *SLCO1B3* 334GT genotypes after MMF administration and found that AUC_{4-12} of the volunteers with *SLCO1B3* GG genotype was (4.79 $\pm$ 1.78) μg·h/ml/g, and (3.80 $\pm$ 1.54) μg·h/mL/g in those with TG genotype, which is 25.9% lower than that of the GG subjects ($P = 0.036$). AUC_{4-12} of subjects carried T allele is 3.63 $\pm$ 1.58 μg·h/mL/g, which is 30.4% lower than that of GG genotype ($P = 0.014$).

   Similarly, Yamakawa et al. [34] studied 34 Japanese patients with chronic myeloid leukemia who received imatinib treatment, and found the serum

clearance rate in patients ($n = 19$) carrying *SLCO1B3* 334GG genotype was (9.5 ± 3.1) L/h vs. (7.0 ± 3.1) L/h in patients ($n = 15$) carrying *SLCO1B3* 334TT and TG genotypes ($P = 0.019$). The serum clearance rate in patients with *SLCO1B3* 334GG genotype was even higher, indicating that the ability of OATP1B3 in transporting drugs into hepatocytes was increased and result in reduced drug exposure.

2. ABC transporters

   (a) *ABCB1* is the most important member of the ABC family, which encodes P-glycoprotein (P-gp). The prime function of P-gp was first discovered in tumor cells. Working as an ATP-dependent influx pump, P-gp prevents accumulation of chemotherapy drugs in cells. It is generally believed that overexpression of P-gp in tumor cells is the main mechanism responsible for acquired drug resistance or multi-drug resistance (MDR) of a group of cytotoxic drugs with different structures. There is also a certain amount of P-gp in normal tissues, which plays an important role in drug absorption and renal/hepatic excretion, and drug penetration of the blood-brain barrier. Sequence variation at exon 26 of *ABCB1* gene is related to P-gp expression in the gastrointestinal tract and the plasma digoxin concentration. In addition, P-gp participates in intestinal transport of tobramycin, azithromycin, and clarithromycin. Genetic polymorphism is related to individual differences in intestinal drug absorption. In addition, P-gp is an efflux transporter of minocycline across the blood-brain barrier. Many drugs such as minocycline are P-gp inhibitors, and therefore this category of efflux transporters plays extremely important roles in pharmacogenomics. [35]

   (b) Multiple resistance protein 2 (MRP2) is encoded by another important ABC family member *ABCC2,* whose most frequently reported genetic polymorphism is C-24T. This SNP is located near the promoter region of *ABCC2,* whose effect of changing the MRP2 activity is probably through inhibiting the expression of MRP2, which further induces PK change of drugs. A study measured PK parameters of the active metabolites of irinotecan in 67 Japanese cancer patients who received irinotecan-based chemotherapy, and found that AUC_{0-24} of irinotecan in *ABCC2* −24CC patients ($n = 22$) was (1.37 ± 0.697) μg·h/mL vs. (1.94 ± 0.793) μg·h/mL in *ABCC2* −24CT and TT genotype patients ($n = 9$), showing a significant difference ($P = 0.0264$). It is clear that *ABCC2* −24 TT genotype reduces the level of MRP2 expression, thus decreasing the amount of drug excretion via the liver and increasing the amount of drug exposure. As a result, AUC_{0-24} becomes even larger [36].

9.3 Pharmacogenomics and TDM in the Personalized Regimen

Pharmacometrics describes and predicts the PK and PD processes by using mathematics and statistical principles [37]. The key work of pharmacometrics is model establishment and simulation. Models are used to describe changes of drug exposure and disposition over time, population mean value, and individual variation of parameters. Based on the model used, new data are simulated according different situations. Pharmacometrics can be extensively applied to new drug research and individualized drug administration. Population PKs (PPK) is a part of pharmacometrics, which focus on the PK parameters.

The goal of rational drug use is to achieve a good therapeutic effect by avoiding the occurrence of adverse reactions. Design of the therapeutic plan on the basis of PK principles is an important means of rational drug use. PPK can play an important role in the design and implementation of individualized drug administration. The PPK parameters are calculated on the basis of data obtained from representative individuals. The more stronger representative of PPK parameters, the better predictive value of individual PK parameters may be obtained. After a drug is decided for a certain individual, the initial regimen of drug administration according to the PPK parameters. After that, individualized PK parameters can be obtained through additional TDM data and patients characteristics. Based on which optimal individualized drug administration regimen can be designed [38, 39].

9.3.1 Design and Optimization of the Individualized Drug Administration Regimen by Using TDM and Pharmacogenomics

9.3.1.1 Design of the Initial Dosing Regimen on the Basis of PPK Study

Usually the PPK model is constructed according to the retrospective or prospective blood-drug concentration data and the pathophysiological data of the patient. PPK parameters and fixed effect parameters of other studies similar to the patient may also be utilized. It should be noted that data reported in the literature are only referential, especially those obtained from different population.

Calculation of the population value of important PK parameters according to the model and covariates that may affect the PK parameters. For instance, for a drug of one-compartment model which is given intravenously, clearance (CL) and the volume of distribution (Vd) are sufficient to describe the PKs. If the drug is given in an extra-vascular mode, an additional parameter for the describe drug absorption: absorption rate constant K_a is needed.

The fixed effects include the pathological, physiological, and genetic factors. The factors are tested as the candidate covariates during model construction.

Theoretically, there are infinite factors that can affect drug disposition. In clinical practice, we can choose factors may have clinical significance. Genetic polymorphisms of drug-metabolizing enzymes and transporters can affect the PK parameters in varying degrees, and further affect formulation of the drug administration regimen.

Initial assessment of the individual PK parameters according to the PPK model and fixed effects, based on which the initial drug administration regimen can be designed.

9.3.1.2 Optimization of the Individualized Therapeutic Regimen

Based on the PPK parameters, drug concentrations from TDM and the covariates (physiological and pathological index, genetic polymorphism, et al.) which proved to have significant impact on the PPK parameters, a Beyas assay can be used to estimate the individualized PPK parameters, and further design or regulate the dose regimen. In the Bayes assay, 1–3 points-drug concentrations of the particular patient from TDM are used to estimate the individualized PK parameters, which can be further used to design the personalized dosing regimen (Fig. 9.3).

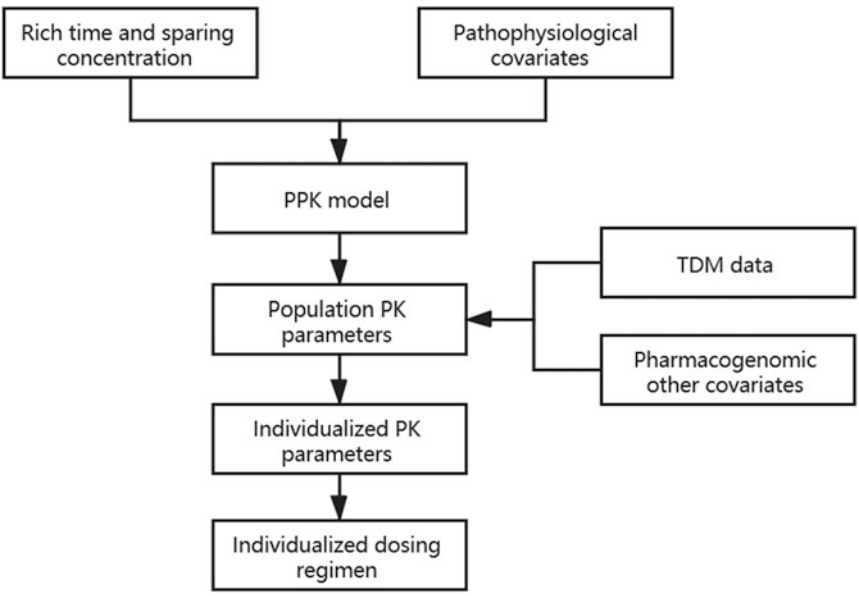

Fig. 9.3 Optimization of individualized therapeutic regimen through a Bayes assay using TDM results

9.3.2 *The Application of Bayesian Assay in the TDM: Role of Pharmacogenomics*

9.3.2.1 Immunosuppressants

Tacrolimus (TAC) is a member of the calcineurin inhibitor family, which blocks the transcription of cytokines (i.e., interleukin-2) and impairs the proliferative of T-cell response. TAC is widely used as an important part of immunosuppressive therapy regimen after organ transplantation. It has been shown there is remarkable inter-individual variation in efficacy and side effect, which is caused partly by highly variable PK characteristics of TAC.

TDM of TAC is considered as a useful tool to achieve an optimum therapeutic effect. Exposure of TAC (AUC) in vivo is proved to correlate well with immunosuppressive therapy outcomes such as acute rejection episodes and chronic rejection. Conventional TDM index trough concentration (C_0) is used to instead of AUC. The C_0 in the 9–13 ng/mL in the first month post-transplantation, and 5–9 ng/mL later is considered as the therapeutic range. Limited sampling strategy (LSS) with 2–4 concentrations is suggested more reliable predictor of AUC. Different TAC PPK models have been established in various population including renal transplant patients, liver transplant patients, hematopoietic stem cell transplant patients, and lung transplant patients.

After administration, TAC is rapidly absorbed in most subjects with peak blood concentration obtained 0.5–1 h after administration. The bioavailability of TAC is about 25% (5–93%) in patients under steady state. Absorbed TAC is extensively metabolized by CYP3A4 and CYP3A5 located in gut mucosa and liver. Over 95% of metabolites were eliminated through bile. Different physiological and pathological factors, including genetic polymorphism of metabolic enzyme and transporters were reported to have remarkable impaction on the PK of TAC. CYP3A5 activity in the human liver and small intestine is strongly dependent on the presence of the CYP3A5∗3 allele. Genetic polymorphisms were also reported to be important in the PKs of TAC. For example, the interaction between TAC and P-gp, which is coded by *ABCB1*, has also been proved. Determination influence of these factors quantitatively is important in the evaluation the inter-individual variation of TAC PKs.

PPK–pharmacogenetic model of TAC is valuable for the prevention of fluctuations in the tacrolimus PKs and C_0 during the early period after transplantation. Different PPK models were established in various populations. Including: patients with varous solid organ transplantation; various ethnic background; patients in various age. The obtained data included rich-time samples or just TAC C_0. Exposure of TAC were estimated by using Bayesian assay in many of the models constructed. In these models, various factors were suggested to have impact on the PK parameters (most frequently CL/F). *CYP3A5* genotypes were suggested as the covariates of TAC clearance (and further AUC) in most of these studies. It was suggested patients with CYP3A5∗1 allele may have a 13–126% faster clearance of TAC (Table 9.4).

Table 9.4 PPK models constructed in organ transplant patients received therapy of tacrolimus

Organ	Population	Number of patients	DATA	data	Covariates	Model	Equation for CL/*F* estimation	Ref
Renal	USA	102	500	Full PK + Sparse sample	CYP3A5, ABCC2	2 CMT	$\frac{CL}{F} = 20.7 \times \left(\frac{Age}{50}\right)^{-0.78} \times 2.03 \times (CYP3A5) \times 1.40 \times MRP2$	[40]
Renal	France	50	289	Full PK + Sparse sample	CYP3A5, BW, HCT	2 CMT	$\frac{CL}{F} = 20.7 \times \left(\frac{BW}{50}\right)^{0.75} \times 2.26^{CYP3A5} + 7.11 \times 1.74^{HCT}$	[41]
Renal	Korea	122	1501	Full PK + TDM C0	CYP3A5, POD	1 CMT	$\frac{CL}{F} = 21.9 \times \lfloor 1 + 0.019 \times (POD - 9.6) \rfloor \times 0.816^{CYP3A5}$	[42]
Renal	Australia Norway	242	3100	Full PK + Sparse sample	CYP3A5, FFM	2 CMT	$\frac{CL}{F} = 811 \times \left(\frac{FFM}{60}\right)^{0.75} \times 1.30 \times CYP3A5$	[43]
Liver	UK	43	628	TDM C0	CYP3A5, POD, BW	1 CMT	$\frac{CL}{F} = 12.9 \times \left(\frac{BW}{13.2}\right)^{0.75} \times e^{(00.00158 \times POD)} \times e^{(0.428 \times CYP3A5)}$	[44]
Lung		78	2184	Full PK	CYP3A5, CF	2 CMT	$\frac{CL}{F} = 22.5 \times (1.4)^{CYP3A5}$	[45]
Renal	Chinese	161	873	TDM C0	CYP3A5, CYP3A4∗1G, HCT	1 CMT	$\frac{CL}{F} = 26.6 \times \left(\frac{HCT}{27.9}\right)^{-0.451} \times CYP3A5$	[46]
Renal	France	22	171	Full PK	CYP3A5, BW	1 CMT	$\frac{CL}{F} = 30.6 \times \left(\frac{BW}{70}\right)^{0.75} \times CYP3A5$	[47]
Renal	Korea	80	1420	TDM C0	CYP3A5, POD, HCT	1 CMT	$\frac{CL}{F} = 22.9 \times e^{(0.17 \times CYP3A5*1/*3 + 0.0525 \times CYP3A5*3/*3)} \times e^{(0.297 \times HCT_{LOW} + 0.117 \times HCT_{HIGH})} \times POD^{-0.0076}$	[48]
Renal	France	41	875	Full PK	CYP3A5, HCT	2 CMT	$\frac{CL}{F} = 20.3 \times \left(\frac{HCT}{35}\right)^{-1.05} \times 0.37^{CYP3A5}$	[49]

Advagraf® is a newly developed prolonged-release tacrolimus formulation. A PPK model was established based on data from two PK trials were carried out. One study consisted of 145 PK profiles, obtained from 32 de novo renal transplant patients. The other study included 41 PK profiles obtained from 41 adult stable renal transplant patients (more than 12 months post-transplantation) converted from cyclosporin A to Advagraf® for more than 6 months before the present study (initial dosage of TAC 0.2 mg kg^{-1} day^{-1} and further adjusted as for the other study).

CYP3A5 polymorphism and hematocrit were significantly associated with TAC apparent clearance. CL/F of TAC in patients carried CYP3A5∗1 allele was 42 l/h, which was twice as high as those patients with the CYP3A5∗3/∗3 genotype (21 l/h). The Bayesian estimator with concentration determined at 0, 1, and 3 h post-dose could estimate tacrolimus AUC accurately (bias = 0.1%) and with good precision (8.6%).

In another study, a PPK model was established in 28 pediatric nephrotic syndrome (NS) patients received therapy of TAC. A one-compartment model and first-order elimination were best fit with the TAC data. Body weight and CYP3A5 genotype significantly affected TAC CL/F. Patients carried CYP3A5∗1 allele had 60% higher CL/F. Monte Carlo simulation was used to achieve the target concentrations of 5–10 ng/mL. The dose of TAC was simulated on a 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 mg/kg twice daily basis according to different CYP3A5 genotype groups. One thousand simulations were carried out using the initial dataset, and steady state C_0 of each simulated subject was calculated. An optimal dosing regimen of TAC was then established based on the median of simulated C_0 in each CYP3A5 genotype group. NS patients with CYP3A5∗3/∗3 receiving 0.10 mg/kg twice daily and those patients carried CYP3A5∗1 receiving 0.25 mg/kg twice daily TAC. The results support a potential benefit for CYP3A5 genotyping before or in the early stage of TAC based immunosuppressive therapy.

9.3.2.2 Anticancer Drugs

TDM is helpful in rational therapy of cytotoxic anticancer drugs. There is great inter-individual PK variability in many cytotoxic anticancer drugs. For most cytotoxics AUC is better correlated to PD end-points than C_{max} at the end of intravenous infusion.

The sources of variability in drug response are multifactorial. Pathophysiology, environment, diet, drug–drug interactions, drug allergies, medication errors, and poor compliance, may all have a profound impact on PKs and/or PDs, thereby affecting therapeutic outcome. A significant proportion of variability in drug response can be attributed to genetic factors. Therefore, the rationale behind pharmacogenetic studies is to investigate genes encoding drug transporters, drug-metabolizing enzymes, and drug targets that can predict the usefulness of a particular drug. The combined use of classical TDM (as a phenotyping approach) and genotyping of drug metabolic capacity is currently considered to be sophisticated

way to individualize the dosage of several drugs for which the clinical effects are difficult to evaluate.

5-Fluorouracil (5FU) is the most frequently prescribed chemotherapeutic drugs for the adjuvant and palliative treatment of patients with cancers of the gastrointestinal tract, breast, and head and neck. As more than 80% of the administered 5FU is catabolized by dihydropyrimidine dehydrogenase (DPD), the role of genetic polymorphism of DPD has been proved to be important. Cancer patients with deficiency of activity of DPD showed variation in 5FU-based chemotherapy. Following the administration of 5FU, these patients have higher risk of suffering from severe toxicity even death. Mutations in the DPD gene (DPYD), including c.1905 + 1G > A (IVS14 + 1G > A) and c.2846A > T (p.D949V). Population studies have shown that the prevalence of a partial DPD deficiency is about 3–5%. On the other hand, TDM of 5-FU is useful tool in the therapy.

A PPK model was develped in 30 cancer patients received therapy of 5-FU [50]. The 5FU was administered via a 2-min intravenous bolus, at 300 mg/m^2 (first dose) and 450 mg/m^2 (second dose), respectively. Blood samples were taken prior to injection and at 5–120 min after 5FU injection from the vein of the other arm. A two-compartment model with Michaelis–Menten elimination was found to be suitable for the data, the mean maximum enzymatic conversion capacity (V_{max}) value was 40% lower in DPD-deficient patients (943 ± 310 mg/h, $n = 26$) compared with controls (1749 ± 380 mg/h, $n = 10$) received 300 mg/m^2 5FU ($p < 0.001$). On the other hand, the AUC of DPD-deficient patients and control patients in 5FU 300 mg/m^2 group were 9.1 ± 4.0 mg·h/L and 6.0 ± 4.1 mg·h/L. The positive predictive value and negative predictive value for V_{max}, calculated from 5FU levels at 60 minutes, were 96% and 88%, respectively. Thirteen of these 20 DPD-deficient patients had been treated with reduced doses of 5FU. Four of these patients suffered with grade 3 toxicity. The average dose of 5FU in DPD-deficient patients with mild toxicity (grade ≤ 2) was 61 ± 16% of the normal 5FU dose ($n = 10$). Whereas patients with grade 3 toxicity treated with on average 74 ± 4% of the normal 5FU dose.

Profound differences in the elimination of 5FU could be detected between DPD-deficient patients and control patients. PK 5FU profiling, using a single 5FU concentration at 60 min, in combined DPD genotyping, may be useful in the reducing severe toxicity.

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Chapter 10
Pharmacomicrobiomics

Weihua Huang and Wei Zhang

Abstract The safety and efficacy of drugs are the key issues in clinical treatment. Classical pharmacogenomics cannot fully explain the individual differences of drug responses. Pertinent studies indicate that intestinal microorganisms are significantly associated with the efficacy, toxicity, and adverse responses of various drugs. The Human Microbiome Project (HMP) has initiated the research of pharmacomicrobiomics, which mainly studies the interplay between drugs and microorganisms, involving drug absorption and metabolism, transport, microbial metabolites, immune regulation, ectopic and migration, etc. The pharmacomicrobiomics is an important extension and supplement of pharmacogenomics. Due to the infancy of pharmacomicrobiomics, it is urgent to clarify the relationship between human microbiome and rational drug use in clinic, which may be an important supplement to the classical pharmacogenomics. It is of great significance to put the research of pharmacomicrobiomics in the main position to fully explain the individual differences in drug responses.

Keywords Gut microbiota · Pharmacomicrobiomics · Pharmacokinetics · Pharmacodynamics · Personalized medicine

10.1 Introduction

Precision medicine refined from personalized medicine by the National Research Council (NRC) in 2011 has greatly attracted attentions from medical researchers and clinicians all over the world. Currently, precision medicine used for the patients' treatments involves the population, lifestyle, and medical history by matching

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_10

clinical data and genetic biomarkers. Actually, precision medicine is often confused with genomic medicine that often utilizes a patient's individual genomic information for the disease treatment. However, precision medicine not only refers to the genetic aspects of the genomics, transcriptomics, proteomics, and metabolomics, but also to the environmental factors such as lifestyle and gut microbiota.

Over the past decades, pharmacogenomics has focused on the researches of the differential response of drugs due to individual genetic variation. Generally, the genetic information has been estimated to explain about 60% of variability in individual drug responses [1]. Therefore, pharmacogenetics or pharmacogenomics is insufficient to explain the different individual drug responses, while other factors such as gut microbiota should be addressed.

Recently, the gut microbiota has been regarded as an additional "organ" that has crosstalk with the host to affect health and disease [2]. The human gut microbiota consists of about 500–1000 different anaerobic bacterial species as well as some other microorganisms [3]. The predominant bacterial genera of human gut microbiota are classified such as *Bacteroides*, *Bifidobacterium*, *Clostridia*, *Eubacterium*, *Escherichia, Fusobacterium*, *Lactobacillus,* and *Peptostreptococcus*, but the alpha- and beta-diversities of which have great individual variations [4]. More and more studies have indicated that gut microbiota had significant effects on the pharmacokinetics and pharmacodynamics [5, 6]. Consequently, human gut microbiome could reflect the individual variation of a specific drug response [7].

Gut microbiota as the contributor and modulator of human phenotypes plays an important role in drug response, which may initiate the development of microbiome-targeting methods pertinent to drug efficacy and adverse responses [8]. Pharmaco-microbiomics has been defined how variations within the human microbiome make the influence on drug absorption, distribution, metabolism, excretion, and toxicity [8, 9]. Unambiguously, gut microbiota has been becoming an essential hotspot in the development of precision medicine and an excitingly attractive aspect to affect drug safety and efficacy [9]. Actually, the effects of gut microbiota in health and disease have extended to almost all the disciplines of medicine as well as a new coming era of precision medicine.

10.1.1 Pharmacomicrobiomics

Pharmacomicrobiomics is a new discipline that mainly explores the interplay between drugs and human microbiome including human-related microbial communities and their genomes. Pharmacomicrobiomics focuses on how microbiome variations influence the pharmacokinetics and pharmacodynamics through activation, enhancement, competition by affecting drug biotransformation and the absorption, distribution, metabolism, or excretion (ADME) of drugs, etc. [9, 10] It is a natural extension of pharmacogenomics and a specific branch area of precision medicine. Pharmacomicrobiomics not only emphasizes the impact of human

genome variation on drug therapeutics, but also highlights the role of human microbiome on drug responses.

10.1.1.1 Gut Microbiome

Unlike the term gut microbiota referring to the assembly of eukaryotic microbes (bacteriophages, archaea, and bacteria) and viruses which live in the human gastrointestinal tract, the term gut microbiome has a great impact on our body and is known as the "second brain" referring to the genomes of the gut microbiota including both the gut microbial genes and gene products [11]. In recent years, this "superorganism" gathered by trillions of microbes has attracted a lot of scientific interests. More than 1000 kinds of microorganisms live on the gut mucosal surface or within the intestinal lumen [4]. The human gut microbiota differs individually in composition and consists of microbes classified to all three domains of natural life, i.e., archaea, bacteria, and eucarya (Fig. 10.1).

Metagenomics has evolved to learn more about the hidden diversity of microbes living with us, especially gut microbiome that encodes about 3.3 million specific genes, which are more than 100 times of the number of genes encoded by the human genome [12]. Therefore, gut microbiome is considered to be the second genome of the human beings. The gut microbial genome, along with the human genome, affects our health in different ways through the interaction with environmental factors. The mutation of human genetic genome among different individuals is only about 0.1%, but the variation of intestinal microbial groups among different individuals can reach 80–90% [13]. Although studies showed that each human being had a virtually unique microbiome in its taxonomy and microbial composition with so much

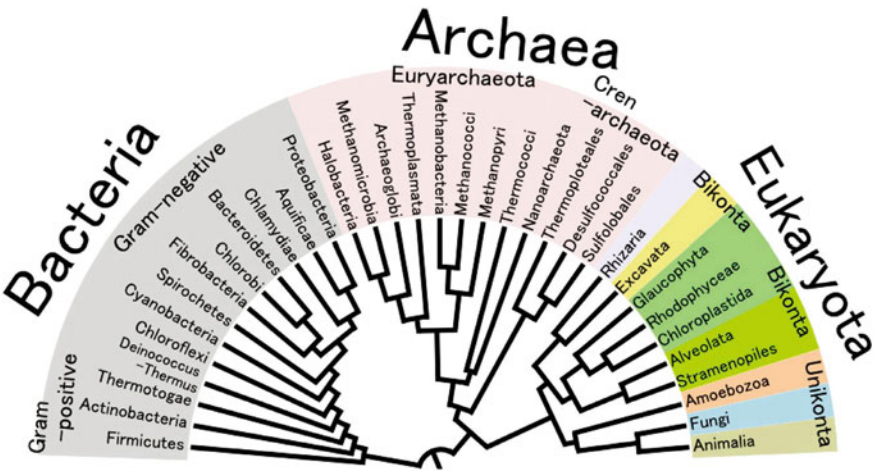

Fig. 10.1 The tree of life according to comparisons of nucleotide sequences in 16S rRNA genes (http://en.wikipedia.org/wiki/File:Phylogenetic_Tree_of_Life.png)

individual variances, the metabolic capabilities and functions carried out by gut microbiota are fairly constant and remarkably stable [14].

Recently, researchers from the European Institute of Bioinformatics identified 2000 new bacterial species and reconstructed 92,143 genomes from the gut microbiota of 11,450 human individuals, which have drawn a new blueprint for the human gut microbial genome [15]. Provided the microbial metabolism and the diversity of microbial coding enzymes, it is vital and crucial to determine whether the difference in individual drug response is associated with the gut microbiome.

10.1.1.2 Development of Pharmacomicrobiomics

In the context of biotransformation and biodegradation of drugs, the interplay between drugs and microorganisms has been studied for a long time [16]. Pharmacomicrobiomics was firstly coined in 2010, which mainly explores the interplay between drugs and microbiome [9]. Compared with pharmacometabonomics and pharmacometagenomics, pharmacomicrobiomics is distinguished by its combination of microbial community composition with drugs. Pharmacomicrobiomics is distinguished by focusing on microbial community composition [9].

The Human Microbiome Project (HMP), especially human gut microbes, investigated how microbial composition and functional changes affected human health and disease [7]. HMP has initiated the study of pharmacomicrobiomics from decentralized observations to systematic research [8]. At the beginning, scientists employed genomic sequencing of intestinal microbes to verify their presence or absence in the gut, the intestinal ecosystem is complex and the symbiotic relationship exists between the gut microbiome and the host [17]. Therefore, it is difficult to commonly establish a "core microbiome" to all healthy people by genomic techniques [14]. It seems that the development of pharmacomicrobiomics has entered the bottle neck period. However, with the continuous development and application of new research methods such as high-throughput sequencing and metagenomics, pharmaceutical microbiology has made new breakthroughs that the composition of intestinal microorganisms varies greatly among individuals, while their functions such as metabolism are relatively different [18, 19].

In the near future, pharmacomicrobiomics will provide a great boost for screening new drugs and designing precision therapeutic drugs. Pharmacomicrobiomics combined with interconnected microbiology, bioinformatics, systemic pharmacology, and toxicology has expanded the scope of precision medicine.

10.1.2 Gut Microbiome of Medicine

It is important and vital to understand the complexity of the interaction between intestinal microorganisms and drugs, and how the presence or absence of specific microorganisms affects the metabolism and efficacy of drugs [20]. Most oral

medications interplay with intestinal microbes in the small and large intestines. Michael Zimmermann et al [21] systematically analyzed the interaction between drugs and microorganisms by measuring the ability of representative 76 human intestinal bacteria to metabolize 276 structurally diverse drugs, as well as identifying drug metabolism-microbial gene enzymes. It is found that these drugs could be bio-converted by at least one type of bacterium, while each bacterial strain is able to metabolize 11 to 95 different drugs [21]. In addition to drug metabolism, gut microbiota can regulate the immune response related to drug therapeutics. For example, Clostridium has been demonstrated to increase primary bile acid content and thereby regulate chemokine-dependent accumulation of NKT cells in the liver [22]. Understanding the effects of genetics, gut microbiota and their interactions on risk factors for various diseases or inter-individual variability of biomarkers will provide insights into host–microbe interactions in health and disease.

10.1.2.1 Human Microbiome Project

The Human Microbiome Program is an extension of the Human Genome Project, which focuses on the relationship between microbial profile diversity and human health through metagenomics. Except the human genome inherited from the parents encoding about 25,000 genes, human beings possess another genome of more than 1000 commensal microorganisms invading into human beings after birth [23]. The genetic information of microbiota is defined as microbiome, or metagenome, which encodes more than three million genes. The two genomes in harmony have crosstalk with each other to affect the health of human beings. Therefore, the study of symbiotic microbes must not be neglected when studying the relationship between microbiota and human health.

In 2007, the National Institutes of Health (NIH) announced the launch of a two-year human microbiome program [10]. The HMP was led by the USA and a number of EU countries, together with more than a dozen countries including Japan and China. The project used a new generation of high-throughput sequencing technology to sequence human microbiome DNA after the completion of the HGP. The goal of this project was to analyze the effects of microbiota on human health by drawing microbial genomic maps in different organs of the human beings [10]. Since the announcement of the HMP, the program is expected to provide an important foundation for significant advances in personalized medicine.

10.1.2.2 Individual Variants of Gut Microbiome

The human gut microbiota is a complex and diverse community that plays a vital role in maintaining the health of the host, but significant variations have been evidenced individually [24]. Understanding the effects of inter-individual variation of gut microbiota on risk factors or biomarkers of various diseases will provide insights into host–microbe interplays in health and disease [25]. A large number of studies

have confirmed the association between the compositional imbalance of the intestinal microbiota and several diseases such as diabetes, obesity, and cancer [26]. Compared with the human genome, the microbiome is more plastic or fluid [9]. The composition and abundance of gut microbes are affected by many factors due to their special environment. Gut microbiome variation occurs not only among individuals, but also within the same individual due to factors such as region space, age, diet, and drugs [9].

The microbiome changes dramatically along with the gastrointestinal tract, with distinct populations in the oral cavity, esophagus, stomach, and small and large intestines [5]. The most abundant genus in the mouth includes *Actinomycetes*, *Neisseria*, *Streptococcus,* and *Verobacter*, while the throat and stomach are mainly *Streptococcus* and *Plasmodium*. In the stomach, about half of people are colonized by *Helicobacter pylori* [5]. The small intestine is usually dominated by genus such as *Peptostreptococcus*, which is rare in the large intestine, and contains fast-growing gram positive organisms that are specifically used to digest simple carbohydrates [27]. However, the large intestine contains most of the microbial group and most of the microbial metabolism occurs at this site [5].

Scientists at the Pacific Northwest National Laboratory and Lawrence Berkeley National Laboratory have used animal studies to correlate specific genes in mice with the presence and density of specific microorganisms in their intestines [24]. They analyzed more than 50,000 genetic changes in mice, and finally found that more than 100 of them affected the number of bacteria in the intestine. Some of these mouse genes are very similar to human genes for diseases such as arthritis, colon cancer, celiac disease, and diabetes [24]. At the same time, the researchers also found that the density of Lactobacillus in the intestines of mice is affected by several genes in mice, and has a significant correlation with the content of helper T cells, which are important immune cells in mice [24]. Due to the complexity of the human body, we lack understanding of the complex interplay between host genetics and early life environment on the microbial and metabolic composition of the gut.

The complexity of the gut microbiota varies with age. It is generally believed that colonization of the intestine begins at birth, while the infant initially receives microbial colonization from the mother as it passes through the birth canal [28]. For example, because the vagina contains a large number of *Lactobacilli*, babies born vaginally contain a large number of *Lactobacilli* in the first few days [29]. Pioneer microbial communities obtained from birth affect colonization of gut microbes. At the early developmental stage, the diversity of the microbiota is usually low, mainly consisting of two major systems, *Actinobacillus* and *Proteus*. Microbial diversity increases with age, and at approximately 2.5 years of age, the composition, diversity, and functional capacity of gut microbiome are similar to adult microbiota [29]. However, the abundance of Bacteroides and Clostridium IV increased in individuals over 65 years of age by comparing with younger subjects [29]. At the same time, studies have shown that the gut microbiome of young people and 70-year-olds are highly similar, but significantly different from the microbiota of centenarians [30]. Without the influence of other factors, the main variations in

microbial communities may explain some differences in drug responses between children and adults.

The emergence of three enterotype divisions shortly tends to form a favored community structure by assembling groups of species. It is found that long-term food intake habits are related with intestinal gut microbiota type. Foods enriched in protein and animal fat are associated with the intestinal form of Bacteroides, while foods enriched in carbohydrates and monosaccharides are associated with the intestinal type of the Bacterium [31]. Ruminococcus enterotype is largely unaffected by foods. The effects of dietary diets on the microbiome from rural areas in Europe and Africa display that the European microbiome is rich in taxa belonging to the genus Bacteroides, while the African microbiome is rich in the genus Bacterium [31]. Gut microbes are not even similar between fraternal twins (they share only half of the genes) [32]. The variances could be repeated in different mice with no genetic differences [32]. Generally, the diet may possess more significant effects than genetic variation on the composition and abundance of gut microbes.

Drugs, especially antibiotics, as well as metformin and proton pump inhibitors, have a dramatic impact on the microbiome and are one of the main causes of variants between individuals [5]. Many other factors such as sex, body mass index (BMI), sleep, and exercise can make differences within groups, but cannot be used to classify individuals [6, 33]. Although the microbiomes of monozygotic twins are more similar to one another than dizygotic twins, some specific components of the microbiome are highly heritable and correlated with phenotype.

10.1.2.3 Gut Microbiome and Personalized Medicine

After the completion of HGP in 2003, many scientists have realized that deciphering human genome genes does not fully grasp the key issues of human disease and health, because human beings have little knowledge of the vast number of microbial floras which have evolved together with their symbiotic mammalian hosts for millions of years in their bodies. Human genetic variation accounts for a small proportion of the coding gene sequence [5], and this small variation does not fully explain the huge phenotypic variation observed among human beings. Studies have shown that dysbiosis, which results in alterations in both structural and functional profiles of the human microbiota, is the major contributor to the pathogenesis of immune, infectious, and metabolic disorders. For example, when the host types of food intake change the metabolism of gut microbes, the apparent genetic characteristics of the host organization were regulated [34]. Moreover, gut microorganisms can affect the amount of warfarin by affecting the absorption of vitamin K2 and the efficacy of digoxin [35]. Clostridium increased primary bile acid content and thereby regulated chemokine-dependent accumulation of NKT cells in the liver [22].

Changing the inherited genes is difficult, but altering microbes in the human body is easier. Gut microbiome is becoming an important part of the development of personalized medicine, the regulation of gut microbiota may be a very attractive method to manage drug efficiency and safety at the individual level [6]. Applying the

most cutting-edge genomics research refers to the fields of disease diagnosis, health management, and precision medicine. From the beginning of healthy examination, the individualized treatments plan correlates with microbial detection and differential microbiota analysis.

10.2 Gut Microbiota of Pharmacomicrobiomics

10.2.1 Drug Biotransformation

Gut microbes are essential for the metabolism of drugs. They metabolize drugs into metabolites of different activity, toxicity, and fates. The chemical mechanism is often different from human enzymes. In the past, little was known about gut microbes, but with advances in technology, some scientists began systematic research on related mechanisms, and made a series of important progress. Most oral medications can enter the large intestine through the upper digestive tract and small intestine in which they encounter thousands of microorganisms that live in the human intestine.

Direct microbial effects on drug response are the chemical transformations of drug compounds by gut microbiota that influence bioavailability or bioactivity of drugs. The chemical modification of intestinal bacteria is very different from that of the liver. Enzymes in the liver usually undergo oxidation and binding reactions, while intestinal microbes mainly undergo hydrolysis and reduction reactions to metabolize xenobiotics [1] (Fig. 10.2; Table 10.1). These drug metabolites are either delivered to the target tissue, or excreted into the intestinal lumen through the biliary system or into the urine by the kidneys [36]. Recently, the effect of intestinal microbiota on the biotransformation of drugs and its clinical consequences have explained the differences of pharmacokinetics in healthy volunteers [1, 21].

10.2.1.1 Prodrug Bioconversion

As a prodrug, prontosil is an antibacterial drug and one of azo dye series examined by Gerhard Domagk for possible effects on hemolytic streptococcal infection. Subsequent studies found that prontosil had no antibacterial effect in vitro, while its activity is due to the decomposition of azo bonds by bacterial azo reductase and the release of sulfonamides with antibacterial activity [1]. Afterwards, a series of active prodrugs, such as sulfasalazine, containing azo bonds and requires biotransformation, have been developed. Bacterial cleavage of the azo bond in sulfapyridine allows specific release of anti-inflammatory sulfapyridine and 5-aminosalicylic acid in the intestine [37].

Lovastatin is an inactive prodrug that requires the hydrolysis of the lactone ring into an open-chain β-hydroxy acid derivative in vivo to inhibit the synthetic enzyme of cholesterol, while gut microbiota can mainly metabolize it by hydrolysis and

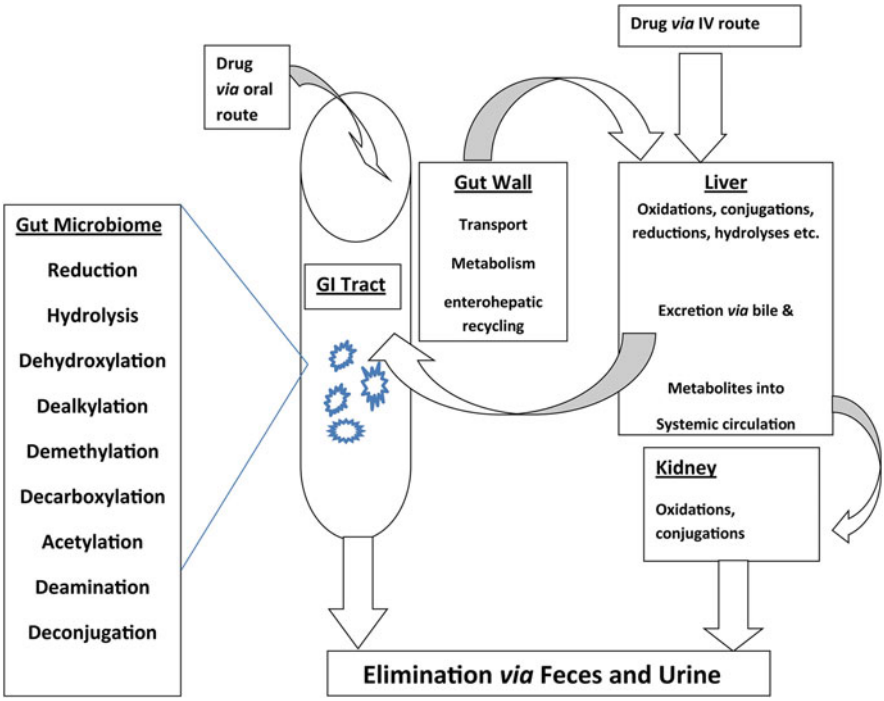

Fig. 10.2 Sites and types of metabolism for drugs after oral or intravenous administration (Figure permitted by Elsevier Publisher) [1]

reduction [38]. One study has shown that antibiotics could reduce the bioavailability of lovastatin, which further proved that intestinal microbes mediate the metabolism of lovastatin [38].

10.2.1.2 Drug ADME

The effects of gut microbiota on pharmacokinetics can happen on the four steps, namely absorption, distribution, metabolism, and excretion. Intestinal microorganisms may affect drug transport by affecting the transporters in the intestine, but there are few reports. Thus, we here mainly describe the effects of gut microorganisms on drug absorption and metabolism.

Gliclazide, a sulfonylurea used to treat diabetes, can be enhanced on its absorption by the use of probiotics [28]. In diabetic rats, the gliclazide content (75 mg/kg) in the blood was higher than that in the untreated rats after 3 days of probiotic treatment, indicating that intestinal microbes might mediate the extent of drug absorption. In a recent study, it is found that taking lactic acid bacteria K8 for three consecutive days could regulate the intestinal microbial enzyme activity and reduce the absorption of oral acetaminophen in mice [39]. This effect may be due to

Table 10.1 Representative drugs/drug metabolites biotransformed by gut microbiota

Biotransformation	Drug/metabolite	Comments
Reduction	Balsalazide	Azo bond reduction
	Bromazepam	Nitro-reduction
	Clonazepam	Nitro-reduction
	Chloramphenicol	Nitro-reduction
	Digoxin	Double bond reduction
	Eltrombopag	Hydrazone cleavage
	Ipsalazide	Azo bond reduction
	Levosimendan	Hydrazone cleavage
	Loperamide oxide	N-oxide reduction
	Metronidazole	Nitro-reduction
	Misonidazole	Nitro-reduction
	Neoprontosil	Azo bond reduction
	Nitrazepam	Nitro-reduction
	Nizatidine	N-oxide reduction
	Olsalazine	Azo bond reduction
	Omeprazole	Sulfoxide reduction
	Potassium 1,2,3,4-tetrahydro-2,4-dioxo-1,3,5-triazine-6-carboxylate (potassium oxonate)	
	Prontosil	Azo bond reduction
	Ranitidine	N-oxide reduction
	Risperidone	Benzisoxazole ring reduction
	Sulfasalazine	Azo bond reduction
	Sulfinpyrazone	Sulfoxide reduction
	Sulindac	Sulfoxide reduction
	Zonisamide	Benzisoxazole ring reduction
Hydrolysis	Azetirelin	Proteolysis
	Calcitonin	Proteolysis
	Diclofenac glucuronide	Hydrolysis to diclofenac
	Indomethacin glucuronide	Hydrolysis to indomethacin
	Insulin	Proteolysis
	Irinotecan metabolite SN-38 glucuronide	Glucuronide hydrolysis
	Ketoprofen glucuronide	Hydrolysis to ketoprofen
	Methotrexate	Production of 4-amino-4-deoxy-N10-methylpteroic acid
	Sodium picosulfate	Desulfation to 4,4-′-dihydroxy -diphenyl-(2 pyridyl)-methane
	Sorivudine (1-beta-D-arabinofuranosyl −5-(E)-(2-bromovinyl)uracil)	Hydrolysis to (E)-5-(2-bromo-vinyl)uracil94
Deacylation	Bucetin	Formation of phenitidine
	Phenacetin	Formation of phenitidine
	Acetaminophen (paracetamol)	Formation of p-aminophenol

(continued)

Table 10.1 (continued)

Biotransformation	Drug/metabolite	Comments
Demethylation	Methamphetamine	N-Demethylation
	4′-hydroxy methamphetamine	N-Demethylation
O-Dealkylation	Fostamatinib	O-Demethylation of the metabolite R529
Dehydroxylation	Fostamatinib	Dehydroxylation of the metabolite R529
	L-Dopa (levodopa, L-3,4-dihydroxy-phenylalanine)	Dehydroxylation
Decarboxylation	L-Dopa (levodopa, L-3,4-dihydroxy-phenylalanine)	
Deamination	5-Fluorocytosine	Deamination to 5-fluorouracil
Oxidation	Levamisole	Thiazole ring-opening
	Lovastatin	Hydroxylated metabolites
Acetylation	5-Aminosalicylic acid	Production of N-acetyl-5-amino salicylic acid
	Sulfapyridine	Production of N-acetyl -sulfapyridine

the fact that probiotics can significantly increase sulfate and aryl sulfate transferases and reduce the beta-glucuronidase which catalyzes acetaminophen metabolism. In addition, the plasma level of amiodarone is increased by 43% after taking probiotics in rats. This implies that the drug absorption is increased by gut microbiota [39].

The effect of intestinal microorganisms on drug metabolism has been extensively investigated. Digoxin used to treat heart failure and arrhythmias is inactivated by the actinomycete *Eggerthella lenta* in the intestine, the metabolism of which could be inhibited by increasing consumption of dietary protein in germ-free mice [40]. Amlodipine, a drug used to treat high blood pressure and coronary artery disease, could be metabolized by gut microbiota. This is why the level of amlodipine in rat plasma could be elevated by co-administered ampicillin which could reduce gastrointestinal microbial transformation [28]. Additionally, the antithrombotic effect of aspirin appears to be also influenced by gut microbiota [41]. The antineoplastic drug doxorubicin is effectively metabolized by *Raoultella planticola* which could hydrolyze doxorubicin into its metabolites 7-deoxydoxorubicinol and 7-deoxydoxorubicinolone via a reductive deglycosylation mechanism [41].

10.2.2 Drug Therapies

Gut microbiota has been demonstrated to affect drug therapies, but little of which was known in the past. With the implementation of HMP, the drug dosage, toxicity,

and drug resistance affected by the gut microbiota have been investigated further, which will guide the assessment of the toxicological risk, and eventually promote the development of precision medicine.

10.2.2.1 Drug Dosages

The gut microbiota can affect the dosage of drugs. The antibiotic users have much higher INR values indicating the higher risk of bleeding after taking warfarin, because some gut bacteria synthesizing vitamin K are killed [42]. It is well-known that vitamin K is involved in the synthesis of coagulation factors II (prothrombin), VII, IX, and X in the liver. The dose of warfarin should be correspondingly reduced according to the reduction of coagulation factor. Otherwise, the risk of bleeding will be increased due to the relative excessive dose. The positive exhalation test of lactose hydrogen shows that the bacteria in the small intestine grow vigorously, while the corresponding dose of warfarin is twice as high as that of the patients with negative index [35, 42]. Therefore, intestinal bacteria can affect the dose of warfarin by affecting the rate and absorption of vitamin K [42].

10.2.2.2 Drug Resistance

Once drug resistance occurs, the efficacy of drugs is significantly reduced. Digoxin, the active substance of digitalis, is used for congestive heart failure and arrhythmia with more than 10% of patients having drug resistance [43]. The feces of drug-resistant person have a higher concentration of the inactive metabolite 2-OH digoxin. Further research finds that in vitro co-culture of *Eggerthella lenta* could reduce the concentration of digoxin, while the use of broad-spectrum antibiotics could increase the concentration of digoxin [2, 40]. By comparing genome sequencing, it is found that the flavin-dependent reductase cgr-2 of *E. lenta* could metabolize digoxin to inactive 2-OH-digoxigenin [40, 43].

Levodopa for Parkinson's disease is interfered by *Helicobacter pylori* and prevented from entering the bloodstream. Because *H. pylori* destroys the duodenal mucosa which is the main site of absorption of levodopa [38]. Moreover, local inflammation and increased release of reactive oxygen species caused by *H. pylori* can inactivate levodopa [38]. Therefore, *H. pylori* treatment can improve the absorption and efficacy of levodopa in patients with Parkinson's disease.

10.2.2.3 Drug Efficacy

Metformin is widely used in the treatment of type 2 diabetes mellitus, but the underlying mechanism is still not well-known. Some studies have shown that gut microbiota is the functional target of metformin [44]. After treating gut microbiota in rats with broad-spectrum antibiotics, the anti-hyperglycemia, the high-fat resistance,

the improvement of insulin resistance, and the improvement of lipid deposition in liver are all decreased. It further proves that the gut microbiota play an indispensable role in the drug efficacy of metformin [44].

There is also a very close relationship between the gut microbiota and the most popular drug immunosuppressants in tumor therapy. For example, in the treatment of MCA205 sarcomas with CTLA-4 antibody, the growth of sarcomas in normal SPF mice is controlled, while that in GF mice and pseudo germ-free mice modeled by antibiotics is invalid [45]. The biggest difference between the two groups is the presence or absence of intestinal bacteria. Furthermore, the abundance of *Fragile Bacillus* in drug-resistant and sensitive patients is proportional to the curative efficacy and the specific *T*-cellular immunity induced by vulnerable *F. Bacillus*. Therefore, the anticancer effect of CTLA-4 antibody is related to the abundance of *Bacteroides fragilis* [45]. Consistent with the previous one, the efficacy of the PD-L1 inhibitor for the treatment of melanoma is directly associated with the abundance of three bacteria such as *Bifidobacteria* in the patients, which can be used as a predictor of efficacy [46].

10.2.2.4 Drug Toxicity

Toxicity occurs when the bacterial transformation of a drug leads to the generation of metabolites that have harmful effects on the host. One of the most famous examples is that CTP-11 (also known as irinotecan), a common colon cancer chemotherapeutic drug, can cause severe diarrhea in up to 80% of users under the action of bacterial enzyme β-glucuronidase [47]. CPT-11 is mainly metabolized by human carboxylesterase in the liver to produce a cytotoxic metabolite SN-38 which inhibits DNA replication. SN-38 could be metabolized to its inactive form, SN-38G, by UDP-glucuronyltransferase (UGT) in the liver. SN-38G is excreted into the intestine through the bile duct and reactivated to SN-38 in the gut by bacterial beta-glucuronidase, while SN-38 is toxic to intestinal epithelial cells and causes diarrhea [47]. Similarly, the bacterial colonization of the glucuronidase can also lead to the toxicity of non-steroidal anti-inflammatory drugs (NSAIDs), which can result in the stomach-duodenal mucosal injury up to 50% of patients [48].

The acetylcholine esterase inhibitor, tacrine, has obvious hepatotoxicity and can be induced to increase the transaminases [49]. Metabolomic studies implicate variations in gut microbial activities pertinent to tacrine-induced transaminitis. Meta-omics analysis indicates that the patients with severe adverse effects have stronger ability to remove glucuronic acid on differential gut microbial composition (e.g., Lactobacillus, Bacteroides, and Enterobacteriaceae) and approximately 9% higher β-glucuronidase gene abundance by comparing with non-responders [49]. Further research discovers that co-administration with oral β-glucuronidase derived from *Escherichia coli* significantly increases the susceptibility to tacrine-induced transaminitis in vivo, but pretreatment with vancomycin and imipenem shows adverse consequences. Therefore, gut microbiota influences the hepatotoxicity of tacrine [6, 49].

10.2.3 Gut Microbiota Intervention

Generally, gut microbiota can be roughly divided into three categories, beneficial bacteria, harmful bacteria, and neutral bacteria. Human health is closely related to gut microbiota. However, gut microbiota dysbiosis is correlated to a variety of diseases, such as obesity, diabetes, liver disease, enteritis, etc. [50] Therefore, it is of great significance to maintain the balance of gut microbiota in health. At present, the common intervention methods are probiotics and prebiotics, fecal microbiota transplant, and antibiotics [51, 52].

10.2.3.1 Probiotics and Prebiotics

Most of probiotics are lactic acid-producing bacteria, especially belonging to the genera *Lactobacillus* and *Bifidobacterium*, other genera such as *Bacillus*, *Enterococcus,* and *Streptococcus* with some concerns regarding their safety, since some strains of these genera are potentially pathogenic. Most of probiotics are lactic acid-producing bacteria, especially belonging to the genera *Lactobacillus* and *Bifidobacterium* [3]. Other genera are also used, such as *Streptococcus*, *Bacillus,* and *Enterococcus*, but some strains of these genera may be pathogenic, so their safety needs to be considered [3]. Moreover, saccharomyces yeasts have also been used as probiotics. A large number of studies have shown that probiotics had beneficial effects on asthma, eczema, obesity, metabolic syndrome, gastrointestinal diseases, and so on [53]. Several mechanisms are supposed to be responsible for the beneficial effects exerted by probiotics [54]: (1) interaction with colonocytes and maintenance of the intestinal barrier; (2) production of antimicrobial factors (such as H_2O_2, bacteriocins, defensins, short-chain fatty acids) which suppress pathogen growth; (3) competition for adhesion and nutrients with potentially harmful microorganisms; (4) degradation of toxins; (5) regulation of enzymatic activities in the colon; (6) activation of the immune response.

Prebiotics are defined as non-viable food components that benefit the health of host by selectively promoting the growth/activity of one or few microorganisms in the colon [11]. Prebiotics consist of fiber and carbohydrates that are not digested at the large intestine and fermented by symbiotic bacteria. This fermentation produces short-chain fatty acids (SCFA) that lower intestinal pH value and maintain the growth of gut-friendly bacteria such as lactic acid bacteria and *Bifidobacteria* [11]. Resistant starch (Rs) is one of the most widely studied prebiotics, which promotes bacterial growth and has anticancer and anti-inflammatory effects. Numerous studies have shown that soy fiber can relieve diarrhea and anorexia, while oat fiber and pectin can alleviate the severity of MTX-related enterocolitis. In addition, the combination of probiotics and CPT-11 in colon cancer rats reduces the toxicity of irinotecan, although no correlation is found with specific bacterial populations. But the observed improvement is associated with increased butyric acid production [1].

The combination of probiotics and prebiotics is called synbiotics and has a synergistic effect. In symbiotic preparations, probiotic elements should selectively promote the growth/activity of probiotics [50]. A synbiotics containing *B. breve* strain Yakult, *L. casei* strain Shirota, and galacto-oligosaccharides decreases the severity of diarrhea, lymphopenia, and the occurrence of febrile neutropenia caused by the docetaxel, cisplatin, and 5-FU chemotherapy regimen [3]. Probiotics and prebiotics benefit human health by affecting the composition and function of gut microbes, while studies have shown that individual differences in gut microbes are associated with the efficacy of probiotics and prebiotics [50]. So personalized strategies are necessary for the success of probiotics and prebiotics biotherapies.

10.2.3.2 Fecal Microbiota Transplant

Fecal microbiota transplantation (FMT) is a method of therapeutics by reconstructing the gut microbiota [54]. Nowadays, FMT often focuses on the treatment of *Clostridium difficile* infection [51]. Successful cases inspired many investigations pertinent to FMT, which has been employed to treat many diseases such as inflammatory bowel disease, irritable colon syndrome, chronic constipation, and non-alcoholic fatty liver disease, especially pseudomembranous enterocolitis due to *C. difficile*. FMT brings new therapeutic approach to diseases that are difficult to manage with conventional treatment.

In order to minimize the risk of the new pathogen, the donor of FMT should be usually selected from the patient's wife, close relatives, or neighbors. According to relevant statistical analysis, the proportion of donors who are close relatives (93%) are slightly higher than that of outsiders (84%), but there is no statistical difference between these ratios [51]. So the most important thing is to determine who has healthy gut microbes. If we believe that our gut microbiota is a person-specific organ just like a fingerprint, the treatment of microbial diseases requires the consideration of individual differences [52]. The issues of FMT refer to age, donor selection, post-transplantation process management, environmental factors, especially the diseases that recipients and donors carry, amount of drug administrated, and content to be transplanted, etc. [52].

10.2.3.3 Antibiotics

Antibiotics have been widely used in the treatment of human and animal infections. Antibiotics naturally exist in all microbial communities as part of the natural lifestyle of bacteria because they produce antibiotics to compete and survive in multiple microbial communities [33]. Rational mechanisms for antibiotic treatment include reducing bacterial density, eliminating targeted harmful bacteria, inhibiting secondary bacterial proliferation, and reducing bacterial translocation.

Antibiotics have a strong effect on intestinal microbes and the entire bacterial community, which means every bacterium in the microbiota may be affected by

antibiotics, whether they are pathogenic or not [33, 50]. A follow-up study explores the effects of antibiotics on the different human gut microbiota to uncover that the antibiotic treatments modify the gut microbiota composition with an abundance/appearance of certain species and a decrease/disappearance of other species [50]. Interference of gut microbiota depends on the type of antibiotic, dose, exposure time, pharmacological effects, and targeted bacteria. Broad-spectrum antibiotics lead to an imbalance between Firmicutes and Bacteroidetes [55]. Specific properties of antibiotics are partially responsible for the shifts in bacterial composition during antibiotic therapy [56].

Each class of antibiotics has different properties and excretion systems, which results in different patterns of alteration in the gut microbiome composition. Because of great variation of gut microbiota among individuals, antibiotic treatment may have a negative effect on another while helping one person. For example, ciprofloxacin at the dosage of 500 mg twice daily for 5 days affects 30% of the gut microbiota, but the magnitude varies from individual to individual [57]. Microbiologists and molecular biologists have evaluated antibiotic resistance at the bacterial or cloned genome level. Scientists use metagenomic sequences to study the diversity and richness of antibiotic resistance genes in human gut microbiota induced by antibiotics. In summary, the effects of antibiotic intervention on the gut microbiota may also vary widely from individual to individual. So how to use antibiotics as a double-edged sword is a question worth pondering.

10.3 Conclusion

In summary, pharmacomicrobiomics is a new medical area that depends on the intersection of microbiology, pharmacogenomics, pharmacogenetics, pharmacology, and personalized medicine. Human microbiome tremendously and individually contributes to phenotypic variations pertinent to pharmacogenomics, systemic pharmacology, and precision medicine. Up to date, it is very difficult to identify the exact bacterial or microbial gene as the biomarker of diagnosis or prognosis for precision medicine. Eventually, together within and between deeper research on humans from all over the world and individuals themselves, human microbiome will be uncovered on the influences of health and therapeutics.

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Chapter 11
Genotyping Technologies in Pharmacogenomics

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Abstract Individual differences in drug effects are largely influenced by genetic polymorphism. Therefore, detection of drug-related gene polymorphisms is critical for achieving gene-directed personalized medicine. This chapter gives a brief introduction to the commonly used methods for detecting gene polymorphisms, focusing on their detection principles and detection technology platforms. By understanding the principles of these genetic polymorphism detection techniques, it is helpful to select an appropriate platform for pharmacogenomics-related research as well as personalized medicine.

Keywords Gene polymorphism · Pharmacogenomics · Nucleic acid detection · Genotyping

11.1 Introduction

Genetic variation is one of the reasons why individuals have different responses to the same drug [1]. For instance, *CYP2C19* gene polymorphism (rs4244285) affects the metabolism of clopidogrel [2] and the proton pump inhibitor omeprazole [3], thereby affecting their efficacy. Besides gene polymorphism, some somatic mutations have become important markers for guiding the use of targeted anti-tumor drugs for individual patients [4]. With the wide application of next generation sequencing technology, more and more drug-related gene mutations were found, and some genetic variants have become effective targets for individualized drugs in clinical applications [5], promoting the advances of precision medicine. Consequently, accurate and reliable tools for detecting genetic variations are required both in scientific research and clinical applications. Currently there are various kinds of methods based on different principles available for genotyping in

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_11

pharmacogenomics, such as allele-specific probe hybridization, allele-specific primer extension, allele-specific enzyme digestion and ligation. Also various detection platforms have been developed for the corresponding methods. Here we briefly introduce some typical genotyping technologies in the case of principles and corresponding detection platforms.

11.2 Principles of Genotyping Technologies

11.2.1 Allele-Specific Hybridization

Hybridization is an intrinsic feature of DNA. Two oligonucleotide fragments can be hybridized into a double-stranded nucleic acid fragment by base pairing under the conditions of suitable ionic strength and an appropriate temperature (Fig. 11.1). This is a reversible process when the temperature rises to a certain extent, the two oligonucleotide strands are denatured into single-stranded nucleic acid fragments. The temperature, at which half of the double-strand DNA denatured, is defined as melting temperature (Tm) of the DNA. The Tm is mainly dependent on the ionic strength of the solution, the concentration of the oligonucleotide, and the sequences of the oligonucleotide. Commonly, one-base mismatched DNA has a lower Tm than the complementary DNA, thus it is difficult to form duplex for the mismatched DNA at Tm of the complementary DNA due to the kinetic instability. Therefore, the single-base difference between alleles in SNPs could be distinguished on a suitable temperature via detecting hybridization behaviors. Many strategies were proposed for monitoring the hybridization behaviors, such as measuring fluorescence signal changes of double-stranded chimeric fluorescent dyes [6] and fluorescent probe hybridization [7]. Accordingly, many technology platforms based on the principle of allele-specific hybridization were developed, such as high resolution melting curve analysis (HRM) [8], SNP array [9], molecular beacon-based SNP detection [10]. The key point of allele-specific hybridization-based technology is how to ensure the high specificity of the detection. Although single-base difference in nucleic acid sequences results in different Tm values, in some cases the difference in Tm values is so small that it is difficult to ensure the specificity of hybridization. Therefore, the detection conditions should be carefully optimized and precisely

Fig. 11.1 Hybridization with complementary DNA or mismatched DNA

controlled, and sometimes the additional additives (dimethyl sulfoxide, betaine, formamide, etc.) or special modifications (locked nucleic acid, peptide nucleic acid, etc.) to the probe are required to improve the specificity of hybridization, which increases the complexity of the experimental setup.

11.2.2 Endonuclease Digestion

An endonuclease is an enzyme that can cleave the nucleotide chain into two parts. Endonucleases usually recognize certain sequences in the nucleotide chain or some special structure formed by oligonucleotides. The specificity of some endonuclease is high enough to distinguish one-base difference in the recognition sequences or structures, so we can use those endonucleases to develop SNP detection assay. The typical genotyping methods based on endonuclease are Invader assay and restriction fragment length polymorphism (RFLP) technology.

Invader assay is depending on flap endonuclease 1 (FEN1) to recognize an invasive structure, which is formed by an upstream probe invading one base into the double-strand region of downstream probe hybridizing to a target DNA (as shown in Fig. 11.2), and cut the 5′-flap fragment from the downstream probe. Because the Tm value of the downstream probes is close to the reaction temperature, the cleaved downstream probe will dissociate from the template and an intact downstream probe will again hybridize with the template to form an invasive structure, triggering a cycle of cleavage. The cleaved flaps can be captured by hairpin fluorescence reporter probe to form invasive structure again, which is recognized by FEN 1, leading to the cleavage of 5′-end of the hairpin probe. The hairpin probe is labeled with a fluorescence reporter molecule and a fluorescence

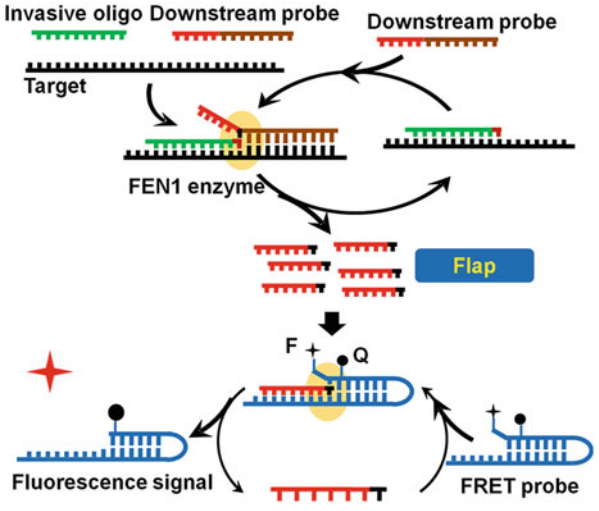

Fig. 11.2 The principle of the Invader assay

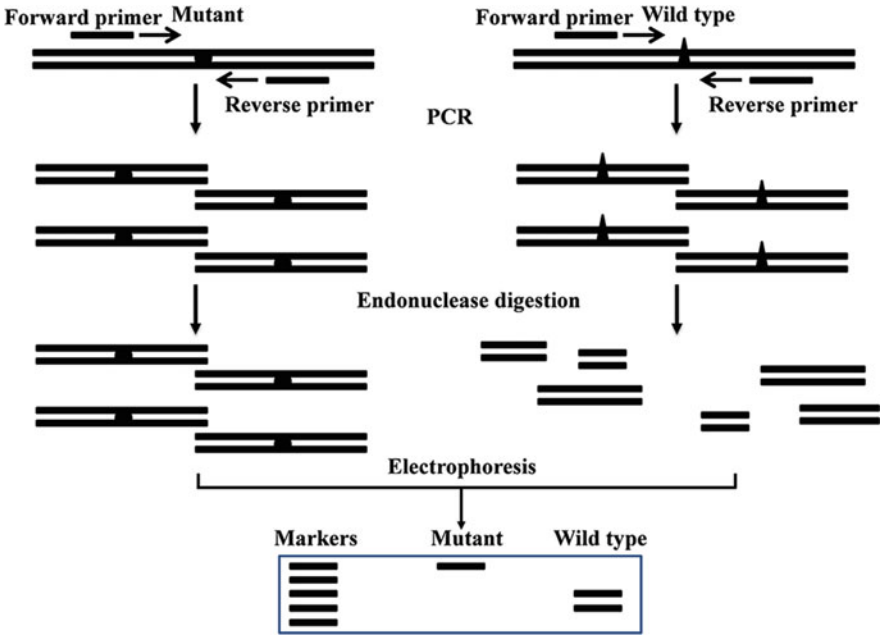

Fig. 11.3 The principle of PCR-RFLP

quenching group at the 5′ area. After cleavage, the fluorescent group on the hairpin probe is separated from the quenching group, generating fluorescence signal. Since the Tm value of flap is designed to be close to the reaction temperature, each flap fragment can be dynamically hybridized with many hairpin probes to form the invasive structure, resulting in gradually enhanced fluorescence signals. The specificity of Invader assay is high enough to identify single-base difference in target DNA especially when the different base is the invaded base in the invasive structure.

Invader assay was successfully used to detect many single nucleotide polymorphisms (SNPs) [11–13], such as factor V G1691A mutation (rs6025) associated with deep venous embolism [14]. In order to achieve quantitative detection, an invasive reaction-based real-time PCR was proposed by employing Invader assay to identify the PCR amplicons and produce fluorescence signals at each annealing step in PCR cycles [15, 16]. Beneficial from the high specificity of Invader assay, the invasive reaction-based real-time PCR is capable to quantitatively detect 0.1% somatic mutations [15] and 0.05% methylated gene fragment [17].

Polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) analysis technique combines PCR and RFLP technology to convert RFLP into PCR products-based markers (Fig. 11.3). Firstly, PCR specifically amplifies the DNA fragments containing the mutant base, and then the PCR products are digested by corresponding restriction enzymes. The principle of RFLP is to detect the size of a particular DNA fragment after restriction endonuclease digestion. Point mutations, insertions, and deletions can lead to new generation and removal of

restriction sites, or changes in the length of the cleavage products [18], resulting in different patterns of the digested PCR amplicons on electropherogram for the homozygous wild type, the homozygous mutant type, and the heterozygous samples.

Although PCR-RFLP is successfully used to determine genetic diseases-related loci (such as cystic fibrosis) [19] and drug-related SNPs [20], the tedious operation and limited types of restriction endonuclease hinder its applications.

11.2.3 Primer Extension

Many genotyping methods are based on primer extension, such as DNA sequencing [21] and allele-specific extension [22]. The specificity of these methods is mainly dependent on the DNA polymerase, which can specifically incorporate correct dNTPs to the 3′-end of a primer fully complementary to the target DNA. By detecting the type of dNTPs incorporated (DNA mini-sequencing) or designing allele-specific primers (allele-specific PCR), a DNA target can be genotyped.

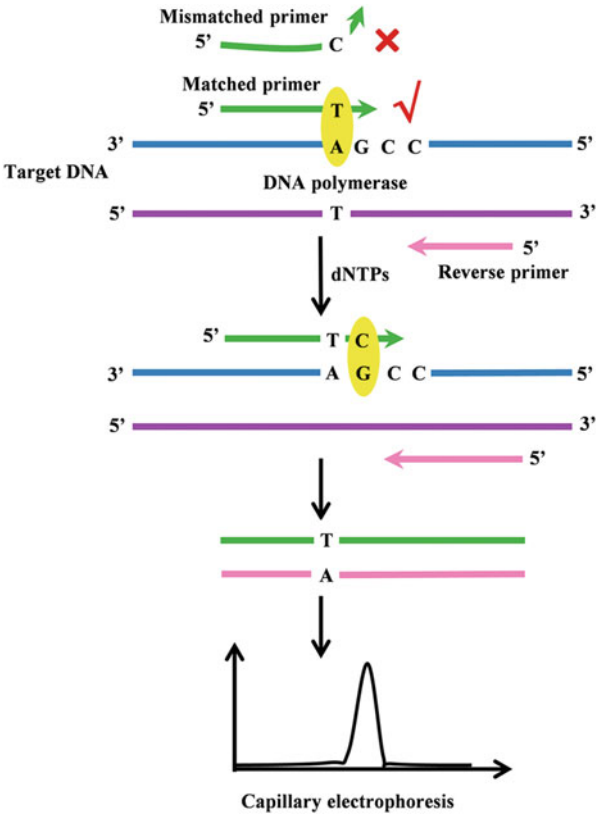

Fig. 11.4 The illustration of primer extension

Most of DNA sequencing methods are based on primer extension (Fig. 11.4). DNA polymerase precisely incorporates a dNTP complementary to target DNA sequence into the 3′-end of a primer. The type of the incorporated dNTP can be identified by measuring the by-product of extension, such as PPi and H+, or detecting the fluorescence signal produced by labeled dNTPs or the extended primer itself. Many technology platforms were used to detect these extension products, for example, DNA chips, DNA sequencers, TOF-MS, and so on.

Besides the high specificity of dNTP incorporation, the recognition specificity of DNA polymerase is also high enough to distinguish one-base mismatch at the 3′-end of the primer. The allele-specific extension method is based on the recognition specificity of DNA polymerase. For SNP detection, two primers, whose 3′-end is respectively complementary to the two base types of the allele, are used. The DNA polymerase only recognizes the matched primer and extends it, thus the allele can be genotyped by measuring extension signals. Allele-specific PCR is the typical technology that uses primer extension to detect DNA mutations.

11.2.4 Allele-Specific Oligonucleotide Ligation

Ligation reaction is another principle widely used in genotyping methods. Ligase catalyzes the formation of phosphodiester bonds between the 5′ phosphate group and the 3′ hydroxyl group of two adjacent oligonucleotides, so the nick of the double-strand DNA can be repaired [23]. The mismatch at the 3′-end or the 5′-end of the adjacent oligonucleotides leads to a very low ligation efficiency. Therefore, we can use this property to design genotyping strategies. There are two typical techniques based on oligonucleotide ligation for gene genotyping: rolling circle amplification (RCA) and multiplex ligation-dependent probe amplification (MLPA).

RCA is an isothermal and efficient enzymatic process driven by unique DNA polymerase (e.g., *Phi*29 or *Bst* DNA polymerase), which can generate long single-stranded DNA (ssDNA) with a circularized DNA as template [24]. According to the amplification model, RCA includes three types: (1) linear RCA (LRCA) reaction with single primer [25]; (2) exponential RCA reaction with multiple primers, including hyperbranched RCA (HRCA) [26] and multiprimed RCA [27]; (3) circle-to-circle amplification [28]. As more than 10^9 copies of amplification product could generate in 90 min, RCA is commonly used for ultrasensitive DNA detection in areas of genomics and diagnostics [29]. For gene genotyping (Fig. 11.5), a long oligonucleotide probe called “padlock probe” is used to hybridize to target DNA, forming a DNA circle with a nick at the end of the probe. The SNP site is located at the 3′-end or the 5′-end of the padlock probe. The matched padlock probe can be ligated by ligase to form an intact circularized DNA probe, which is the template for RCA. The target DNA can be genotyped by detecting the products of RCA with fluorescent-labeled detection probe or fluorescence dye.

MLPA is another genotyping technology based on ligation reaction. In MLPA (Fig. 11.6), a set of probe pairs is used to hybridize to different SNP sites. Each

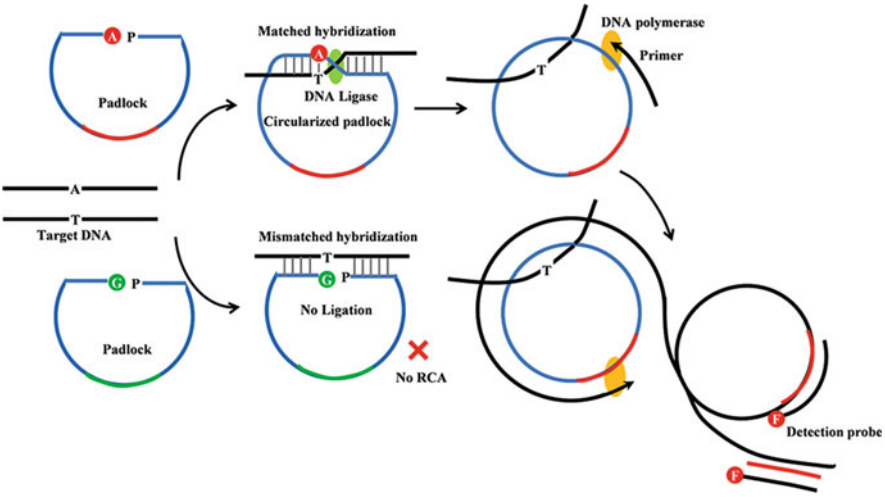

Fig. 11.5 Schematic illustration of the linear RCA for genotyping

target SNP is designed with a set of MLPA probes containing two adjacent regions of target-specific hybridization sequences [30]. After denaturation, the target DNA could be hybridized with specific MLPA probes, and the adjacent regions of hybridization sequences could be ligated by a thermostable ligase. Thus the ligation probe could be amplified by PCR with a universal primer pair (F and R), resulting in large amounts of amplification products with a unique length (130–480 bp). Then, the amplification products could be further analyzed by electrophoresis according to the unique length. If no target DNA exists, the MLPA probe could not be ligated, leading to the absence of the corresponding signal in the separation peak map of the capillary electrophoresis. The ligation of the MLPA probe is highly specific to discriminate a single-base difference, achieving accurately genotyping. MLPA enables relative quantification for variations of up to 45 SNPs in a single reaction [31].

11.3 Platforms of Genotyping Technologies

11.3.1 Fluorescence Resonance Energy Transfer

Fluorescence resonance energy transfer (FRET) is a mechanism that a donor chromophore may transfer energy to an acceptor chromophore, inducing spectral characteristic change. The efficiency of the energy transfer is dependent on the distance between the donor and acceptor. This property is used to develop research tools in biology and chemistry fields [32]. For genotyping, TaqMan probe and molecular beacon (MB) are commonly used technologies based on FRET.

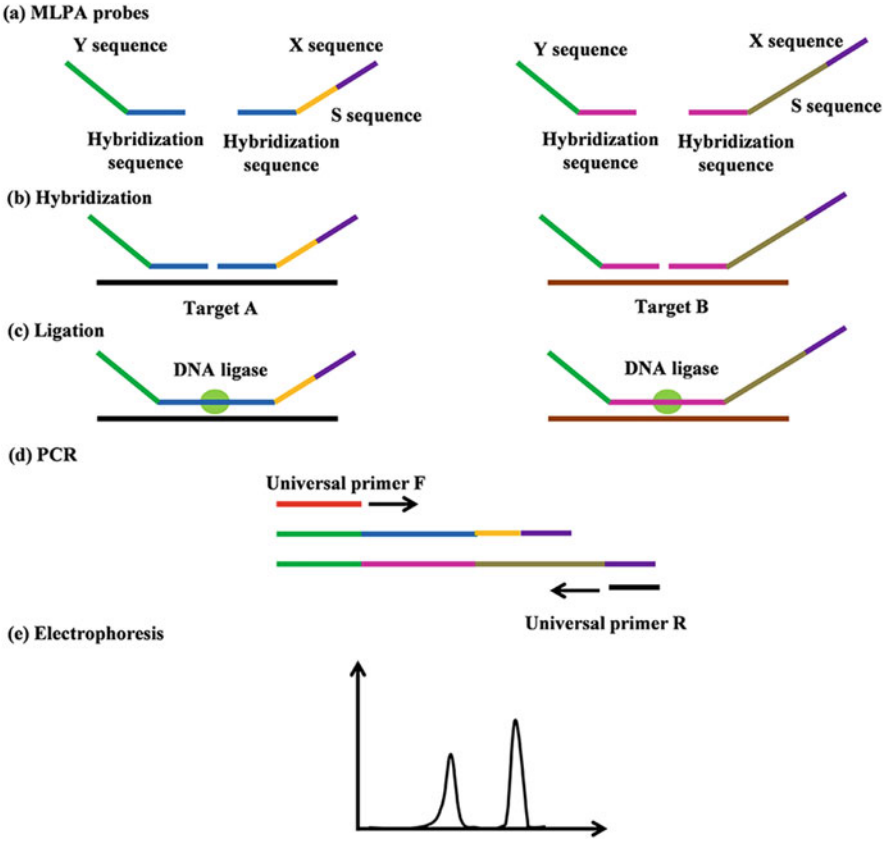

Fig. 11.6 Schematic illustration of multiplex ligation-dependent probe amplification for genotyping. Each MLPA probe consists of two oligonucleotides. One contains the X sequence complementary to the universal primer R and the hybridization sequence specific to the target, and the other contains the Y sequence complementary to the universal primer F, the hybridization sequence specific to the target, and phage M13-derived S sequence

TaqMan probe is a hydrolysis probe designed to increase the specificity of quantitative PCR. The method was first reported in 1991 by researchers at Cetus Corporation, and the technology was subsequently developed by Roche Molecular Diagnostics for diagnostic assays and by Applied Biosystems for research applications. A typical TaqMan probe is an 18–22-bp oligonucleotide labeled with a reporter fluorophore at the 5′-end and a quencher fluorophore at the 3′-end. As shown in Fig. 11.7, during PCR, the probe anneals specifically to the PCR amplicons and DNA polymerase exhibits its 5′ exonuclease activity to cleave the probe, releasing the reporter molecule away from the close vicinity of the quencher, producing fluorescence signals in each PCR cycle. If the sequences of the probe do not match the PCR amplicons, no cleavage occurs, so that the fluorescence signals remain low intensities. The specificity of TaqMan probe is mainly depending

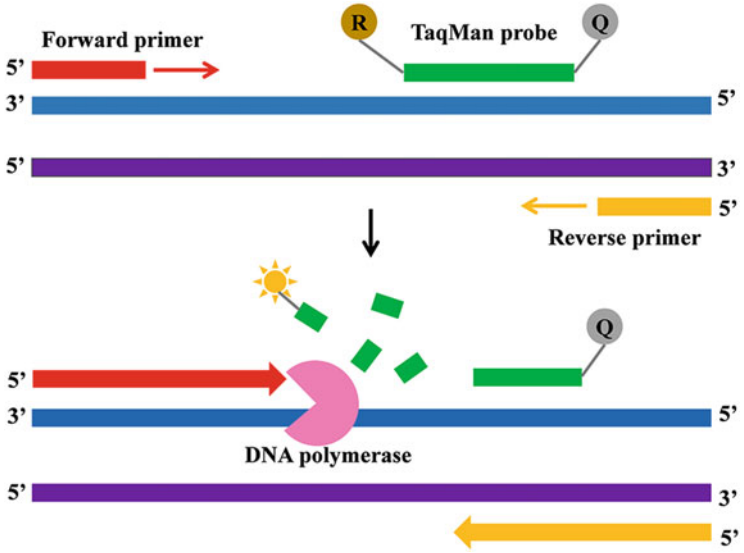

Fig. 11.7 The principle of TaqMan probe-based qPCR

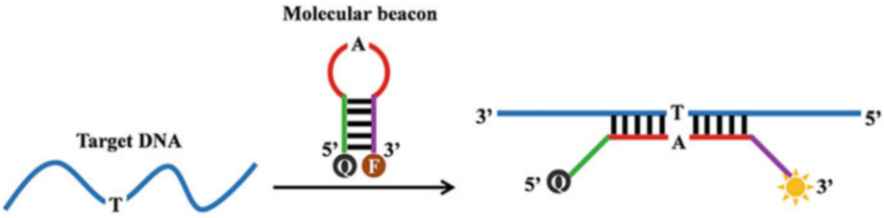

Fig. 11.8 The principle of molecular beacons

on the specificity of probe hybridization. By carefully designing the probe and optimizing the reaction conditions (especially the annealing temperature), a single-base mismatch can be identified. TaqMan probe enables highly specific and close-tube analysis of PCR amplicons, but the design of the probe and the optimization of reaction conditions are usually tediously. In addition, the specificity of TaqMan probe is not satisfied for somatic mutation detection. Therefore, some modified TaqMan probe, such as Minor Groove Binder (MGB) TaqMan probe [33] and locked nucleic acid (LNA) modified probe [34] are usually used to improve the specificity.

MB is a FRET probe containing a single-strand loop and a double-strands stem formed by the hybridization at the 3′-end and 5′-end of the probe (Fig. 11.8). The reporter fluorophore and the quencher fluorophore are at the 3′-end and 5′-end of the probe, respectively, and close to each other due to the stem when no target DNA exists. The sequence of the loop is complementary to a target DNA sequence. When a target DNA hybridizes to the loop, the stem is opened, and the reporter fluorophore

and the quencher fluorophore are separated, producing fluorescence signals. A well-designed MB is sensitive to single-base difference in target DNA, especially when the mismatched base is located in the middle of the loop region [35]. Therefore, MB can be used to detect SNP. Because the reporter fluorophore and the quencher fluorophore are located very closely, the background signals from reporter fluorophore are smaller than TaqMan probe [36]. However, it is difficult for MB to detect somatic mutations as well as TaqMan probe due to the limited specificity of DNA hybridization.

11.3.2 Microarray

Microarray is a DNA sequence variation detection tool that was developed at the end of the last century to meet the needs of large-scale gene function research in the post-genomic era. It has the advantages of high-throughput, simple and convenient operation, easy to achieve automation and low cost, and provides an efficient detection method for high-throughput genotyping.

The principle of microarray is to use an in-situ synthesis or cross-linking method to immobilize tens of thousands of DNA probes onto the surface of the carrier in an orderly manner to produce an array of DNA probes. Then, labeled samples are hybridized to the immobilized probes followed by measuring the intensity of hybridization signals at each probe location to identify the target DNA. There are three strategies for microarray to achieve genotyping.

The first strategy is hybridization-based genotyping microarray [37]. The amplified PCR products containing SNP loci were spotted and immobilized onto amino-modified glass slides to generate a microarray. Then dual-color fluorescence probes specific to different types of the loci are hybridized to the immobilized PCR products (Fig. 11.9). After washing, the fluorescence signals are detected to read out the SNP genotypes. Although this strategy is simple to operate, the specificity of hybridization is strictly dependent on the reaction conditions. Therefore, the false-positive result usually occurs if the reaction conditions are not optimal.

The second strategy is extension-based microarray [38], in which sequence-specific extension of two immobilized allele-specific primers occurs. According to the fluorescence intensity of each spot, we could read the genotype of a sample (Fig. 11.10).

The third strategy is ligation-based microarray [39]. As shown in Fig. 11.11, the discriminating probe (DP) and the common probe (CP) are designed to hybridize adjacently on the template DNA and are joined by ligase in the presence of a matching template. The discriminating 3′-base can be A, C, T, or G. The reaction is thermally cycled and ligation products will be addressed on microarray spots by the unique ZipCode sequences flanking each CP. Hybridization control probe carries a different label (6-FAM) than the DP (Cy3).

One advantage of using microarrays is that they can be designed to detect a wide diversity of genetic variants. As first demonstrated by Cronin in 1996 [40],

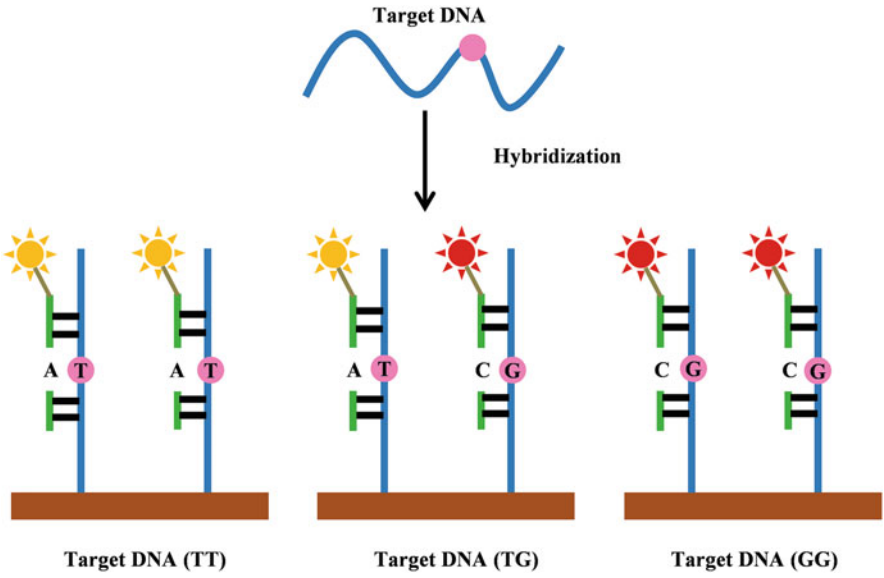

Fig. 11.9 Illustration of the hybridization-based genotyping microarray

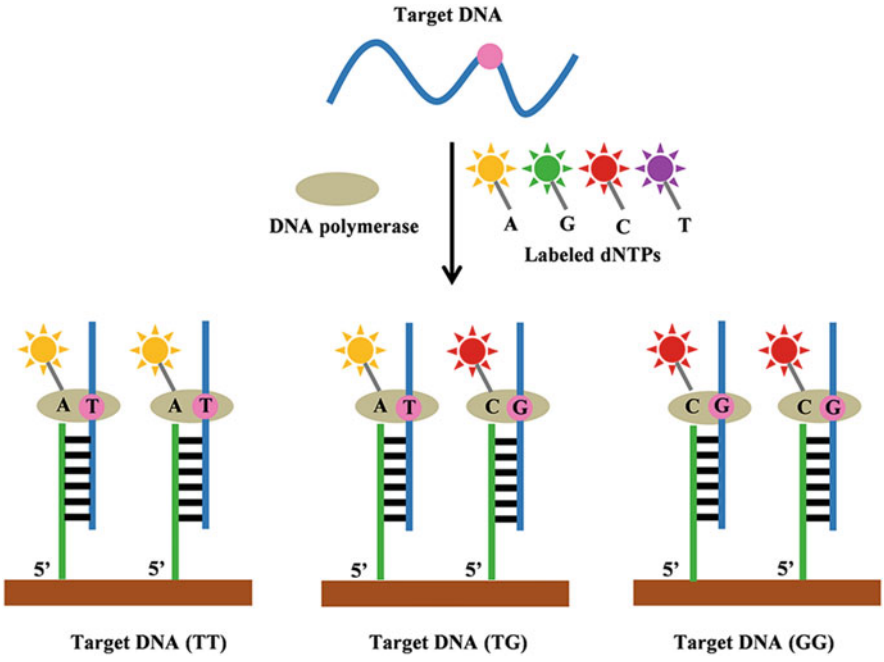

Fig. 11.10 Illustration of the extension-based microarray

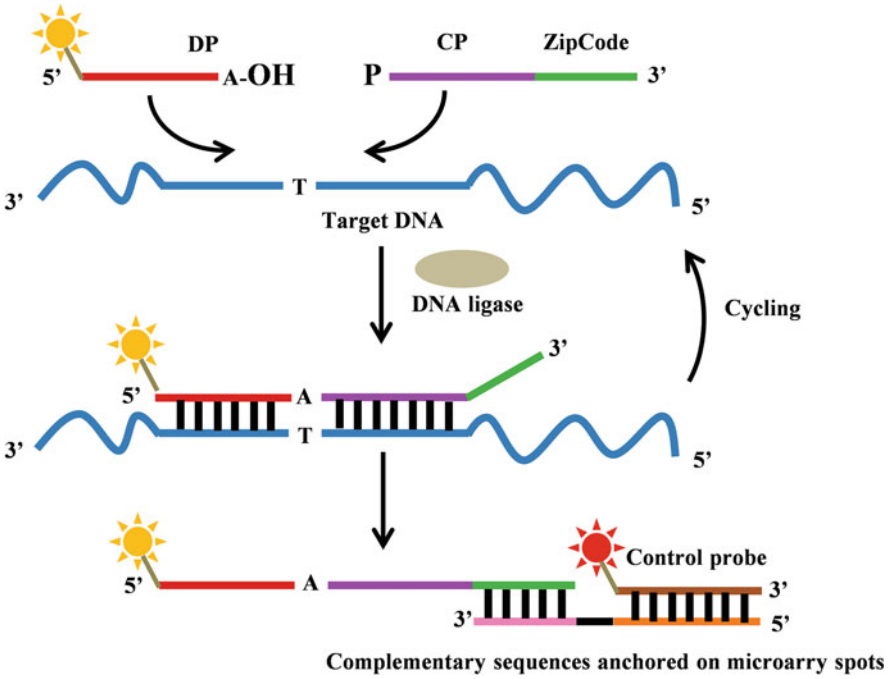

Fig. 11.11 Illustration of the ligation-based microarray

oligonucleotide microarray can readily detect many types of variations. Using a modest size oligonucleotide array containing 1480 probes, the investigators were able to detect known deletions, insertions, and base substitutions in the cystic fibrosis transmembrane conductance regulator gene. Another example, Glas [41] used microarrays to predict the risk for breast cancer metastasis in primary breast cancer samples based on the expression pattern of 70 genes. Furthermore, several microarray-based tests that simultaneously examine variations in multiple genes are approved by the FDA and have entered practice.

11.3.3 Mass Spectrometers

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was initially developed for proteomics application studies, while the full potential of nucleic acid analysis was demonstrated in the year of 1995 [42]. Compared with the detection of proteins, the detection of nucleic acids by MALDI-TOF suffers from some problems, such as matrix or traces alkali metal ions leading to the formation of adducts, low sensitivity and resolution of analysis, unstable molecular ion peaks of nucleic acid molecules, and so on. Fortunately,

with the continuous improvement in sample preparation and purification technology, MALDI-TOF is also widely used in the detection of DNA, especially for SNP detection [43].

MALDI-TOF MS requires biomolecules to form a co-crystal with a suitable matrix. When a pulsed laser (usually 266 nm or 337 nm) is irradiated onto the crystal, the matrix absorbs energy and the co-crystal is volatilized. In the gas phase, a proton transfer reaction occurs between the matrix and the biomolecule, so that the biomolecule is ionized. The ion beam is then accelerated in an electric field of approximately 30 KV, and then passed through an electric field-free drift tube. Although all ions receive the same kinetic energy in the acceleration zone, the speed of each ion differs due to their different mass-to-charge ratios. Therefore, the time of flight (TOF) of each ion that reaches the detector through the drift tube is different. The molecular weight of each ion can be calculated by the TOF.

For SNP detection, the allele-specific product should be firstly generated and then analyzed by MALDI-TOF MS. Four methods are usually used for generating allele-specific products, including primer extension, probe hybridization, restriction enzyme digestion or ligation. The primer extension reaction is the most widely used method to couple with MALDI-TOF MS for SNP detection. In this method, the target DNA is amplified by PCR and then immediately annealed with an extension primer. The 3′-end of the primer is located upstream of the SNP site, and the single-base extension is performed in the presence of four types of ddNTPs. The ddNTP complementary to the SNP site of the target DNA is introduced into the 3′-end of the primer after extension. The extended primers with different molecular weights can be identified by MALDI-TOF MS. In order to improve the resolution and accuracy, additional mass tags can be introduced on the primers or ddNTPs [44].

Primer extension coupling with MALDI-TOF MS is reliable, flexible, and easy to achieve high-throughput multiple SNP analysis. The MassARRAY® molecular weight array system developed by Sequenom (USA) is the only device that directly detects multiple SNPs by mass spectrometry. It can process two gene chips at the same time, and each chip can detect 384 samples × 15 SNP sites.

11.3.4 Sequencing Platforms

Sequencing is one of the most widely used techniques to reveal the function of genes. The development of precision medicine is beneficial from advances in sequencing technology [45]. Sequencing can directly tell us the sequence information of SNP, so it is the gold standard for SNP detection. The main sequencing technology includes Sanger sequencing, pyrosequencing, and next generation sequencing (NGS).

The first working draft of the human genome was completed by using Sanger sequencing technology. In Sanger sequencing process, the primer extension is performed by using four kinds of fluorophores-labeled dideoxynucleotides (ddNTP) mixed with dNTP, thus the extension stops randomly at the different

position on the DNA template, producing the extension products with different length. Then, the extension products are separated by polyacrylamide gel electrophoresis, and the sequence information can be read out according to fluorescence signals and lengths of the products. Sanger sequencing is able to sequence up to 1100 bp of target DNA, but it is unable to detect mosaic alleles below 15–20% [46], which limits its application in somatic mutation detection.

Different from Sanger sequencing, pyrosequencing is based on sequencing-by-synthesis. It employs four enzymes to accurately detect nucleic acid sequences during the synthesis. Four kinds of dNTP are added to the reaction system one by one. If the added dNTP is complemented to the target sequence, DNA polymerase can incorporate the dNTP into the 3′ end of the primer, releasing equal molar of pyrophosphate (PPi). The released PPi is subsequently converted to ATP by ATP sulfurylase and then immediately sensed by luciferase, producing a proportional amount of light. Apyrase is in charge of degrading residual dNTP and ATP to ensure no residual effects on the next dNTP adding. The light signals can be converted to electric signals by photosensitive device, thus the sequence can be detected by measuring the electric signals. Because the signal is proportional to the amount of dNTP incorporated, pyrosequencing can achieve quantitative detection of the target. This property makes pyrosequencing able to detect DNA methylation [47], gene expression [48], and miRNA quantitation [49].

NGS is a high-throughput sequencing technology that emerged in 2005. Several NGS platforms had been developed including 454 FLX pyrosequencing platform, Solexa Genome Analyzer platform, ABI SOLiD sequencer, and Ion Torrent system. The first step of all platforms is fragmentation of the sample DNA, followed by ligation to a common adaptor set for clonal amplification. Sequencing mainly employs two categories: sequencing by ligation (SBL) and sequencing by synthesis (SBS). SOLiD is a ligation-based sequencing platform, while Solexa, 454 FLX pyrosequencing platform, and Ion Torrent system are based on SBS. The advent of NGS has revolutionized biology, genetics, pharmacogenomics research, and the diagnosis and treatment of diseases, leading to the development of precision medicine [50]. Although NGS enables the cost of sequencing one person's genome fewer than 1000 dollars, the preparation of sequencing library and the data analysis are still tedious. Thus, NGS is suitable for high-throughput detecting thousands of SNPs and genome-wide association study (GWAS), not very useful for the detection of a few known SNP sites.

11.4 Conclusion

Genotyping plays an increasingly important role in personalized medicine. At present, more than 100 drug instructions approved by the US FDA indicate that it is necessary to pay attention to the effect of genotype on the drug effects. Therefore, many genotyping technologies have emerged. Although these genotyping techniques are quite different, they are essentially based on four principles: nucleic

Table 11.1 Comparison of genotyping technologies

Genotyping technologies	Principles	Platforms	Advantages	Disadvantages	Suitable application
TaqMan probe/ MB-based real-time PCR	Allele-specific hybridization	FRET	Closed-tube operation	Difficult to optimize the reaction conditions	Genotyping small numbers of SNP loci
Invader assay	Endonuclease digestion	FRET	Closed-tube operation/ accurate	Require special FRET probe	Genotyping small numbers of SNP loci
Microarray	Allele-specific hybridization/ primer extension/ligation	Microarray	High-throughput	Open-tube and tedious operation	High-throughput SNP genotyping
MassARRAY®	Primer extension/ligation	MALDI-TOF MS	High-throughput	Open-tube and tedious operation	High-throughput SNP genotyping
Sanger sequencing/ pyrosequencing	Primer extension	Sequencing platforms	Accurate	Open-tube and tedious operation	Genotyping small numbers of SNP loci
NGS	Primer extension/ligation	Sequencing platforms	High-throughput/ accurate	Open-tube and tedious operation	High-throughput SNP genotyping

acid hybridization, extension, enzymatic digestion, and ligation. According to these four principles, different genotyping detection technology platforms have been developed. Each of these technologies has advantages and disadvantages, and the scope of application varies (as shown in Table 11.1). We need to choose the appropriate genotyping technology for different purposes. For example, high-throughput detection techniques are often required when screening drug-related gene polymorphism sites. Thus, gene chip or NGS is a better choice for high-throughput screening of drug-related gene polymorphism sites. If the number of gene polymorphism sites to be detected is small, real-time PCR or pyrosequencing can be easily achieved. Therefore, understanding the principles of these genotyping techniques will help us to choose a right technology for a right detection target. Among these genotyping technologies, sequencing has the highest accuracy, and the detection results of other methods generally need to be verified by sequencing. Therefore, sequencing has always been the gold standard for genotyping. As the cost of NGS continues to decrease, we believe it will eventually become the most important genetic testing tool in the field of personalized medicine.

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Chapter 12
The Ethical, Legal, and Regulatory Issues Associated with Pharmacogenomics

Liyan Miao, Jingjing Zhang, Ling Yi, and Shenjia Huang

Abstract Developed with more and more implementation of pharmacogenomics (PGx), both in clinics and extended to some testing companies, more and more issues related have arisen, as ever-increasing participants from all parties are included. In this chapter, we tried to look into the issues from ethical, legal, and social perspectives, respectively. The confidence of association between geno-information and drugs, involving their pharmacokinetics, pharmacodynamics, therapeutic effects, etc. could be a crucial factor rises issues when taking PGx tests. Also, the ethnic differences, vulnerable populations were addressed with possible PGx characteristics. The quality of informed consent was discussed, fitting into a picture of "good enough" consent elements as a holy grail and their inevitable conflicts in clinical practice regarding PGx testing, which means both right and responsibility. In addition to ethical prospects, issues relevant to legal problems and general social point of views were mentioned as well, including procedures in whole process of PGx test, individual's data protection, and public attitudes and concerns towards PGx. Besides, guidelines concerning PGx in clinical research and practice were cited and compared, especially to recent rules and regulation issued by China government, which is a representative of developing countries with rapid move of PGx science. It is expected to share our opinions on ethics of PGx, giving rise to more concerns on personal safeguard protection and more humans maximized benefits, both common and individual ones.

Keywords Ethics · Pharmacogenomics · Precision medicine · Regulations

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_12

12.1 Introduction

12.1.1 Development of Ethics

Ethics is generally known as the discipline dealing with what is good and bad and with moral duty and obligation. Medical ethics, as one of the most rapidly developing and controversial disciplines in applied ethics, originated from the Hippocratic oath in the fourth century BC. Its main purpose is that doctors should take measures in favor of patients according to their own "ability and judgment" to protect the privacy of patients. The World Medical Association (WMA), the only international organization that seek to represent all physicians, regardless of nationality or specialty, had undertaken the role of establishing general standards in medical ethics that were applicable worldwide. It enacted the three codes, Declaration of Geneva in 1947, International Code of Medical Ethics in 1949, and Declaration of Helsinki in 1964, which developed the spirit of Hippocratic oath and safeguarded the ethical principles of the medical profession [1]. It was well known that ethical principles such as respect for persons, informed consent, and confidentiality were basic to the physician–patient relationship. However, the application of these principles was not always plain sailing in clinical practice, since healthcare personnel, patients, their family members might disapprove about what was the valid way to act in a situation. In addition, with the development of society, the progress of medical science and technology, the complication of interpersonal relationship, the diversification of health risk factors and the advancement of globalization, the ethical issues involved in medical practice were increasingly complicated [2].

After more than 30 years of development, Chinese medical ethics has gradually formed a group of representative academic organizations and research institutions. The ethical issues still focused on the balance of individual rights and collective rights, conflict of procedural justice and essence justice, controversy of local interest and the overall interests, contradiction of nationality and universality values, issue research and system construction, and so forth. Discussion and exploration on these issues will show the way for Chinese medical ethics research in China [3].

Not only China, but also other countries, the achievements in theory and practice of medical ethics were behind the progress of science and technology, so were the confusions and issues. In the late 1990s, pharmacogenomics (PGx) emerged as a new discipline in the field of drug development [4, 5]. As the human genome project and molecular techniques developed, people gradually realized that the different reactions of different individuals to the same drug were largely due to genetic differences. Finding genetic variations associated with drug reactions in humans can be used to find new drug targets and for drug's safe dosage, and balance efficacy with minimal adverse reactions [6]. Due to potential economic value and great significance to patients, pharmacogenomics had been applied to clinical practice in some developed countries. However, as a new technology, pharmacogenomics detection technology itself and its social impact still have many uncertainties. And each social aspect gives high attentions to PGx, especially in medical staff

and ethicists, thought that the clinical application posed new challenges to traditional biological ethics.

12.1.2 Ethical Principles in the Genomic Era

Practice of pharmacogenomics requires a strong ethical and legal support system as human DNA has always been a sensitive area [7]. The Human Genome Organization (HUGO) was an international organization of over 1300 scientific researchers from more than 50 countries, which was conceived in later April 1988, and incorporated in Geneva, Switzerland. The HUGO ethics committee had a truly international membership, avoiding a USA or European bias, who developed six major statements based on the consensus of all members, covering major ethical aspects of genetic research. The statements involved the principled conduct of genetic research, DNA Sampling and Control, Cloning, Benefit Sharing, Gene Therapy, and Human Genomic Databases, which formed a consensus of multinational character in biotechnology industry and genomic research and was a first step towards international regulation [8].

In the past, HUGO Ethics Committee Statements had promised that the highest ethical priority in the application of genomic knowledge is to save lives and reduce suffering; nowadays, the Committee considered that greater attention to the ethical principles of solidarity and equality should be urgently needed. Ethics in the genomic era also include the following long-accepted ethical considerations: balance of benefits and risks, protection of human rights, the rights of privacy, confidentiality, and avoiding harm [9].

On December 2, 2000, Chinese human genome social, ethical, and legal committee stated that they accepted the principles of HUGO and UNESCO's universal declaration on the human genome and human rights [10]. The statement recognized that the human genome was a part of the common heritage of mankind. People should uphold international norms of human rights, respect the values, traditions, cultures, and personalities of participants, and safeguard human dignity and freedom. According to the relevant principles and documents of HUGO, the committee reached the following consensus on the application of the human genome and its achievements:

- The application of the human genome and its achievements should focus on the treatment and prevention of diseases, should not be used for eugenics.
- The principle of informed consent or informed choice should always be adhered to.
- The privacy of individual genome should be protected, and genetic discrimination should be opposed.
- Efforts should be made to promote equality for all, ethnic harmony and international

As we know, the completion of human genomics project was a great milestone in history, which brought us into a genomic era when the field of PGx has emerged, studying on how to apply genomics in pharmacy field, so as to serve human's general benefits. The applications of PGx, both in research and clinical practice, aim at the common goal of bringing healthier and better quality of life to people. Despite good willingness, different perspectives from all parties involved in pharmacogenomic events may occur conflicting thinking or action, giving rise to issues related.

12.2 General Issues Associated with Pharmacogenomics

There is no exact and definite methodology to distinguish the class of an issue, though generally in public recognition, issues being divided into ethical, legal, and social ones according to their main concerns. In particular, the ethical issues are those in the fields lack of legislation or not having very tight relationship to laws, but more in moral level. Nevertheless, the ethical issues could also be the legal and social ones, as they may arise from some inappropriate rules and regulations, and from different views of people involved or observed, like subject vs investigator, donors vs recipients, executors vs governors, healthy participants vs pharmaceutical fellows, and so on. In this chapter, it is discussed about the main issues in the ethical, legal, and social aspects, never a full list, but to draw and share more thoughts in ethical challenges of PGx.

12.2.1 Ethical Issues

It is known that polymorphisms relevant to drug response may overlap with disease susceptibility, and the divulging of such information could jeopardize an individual and his family. PGx test includes the collection and archiving of sensitive information on individual's genetic makeup, so it raises questions of privacy and security as well as ethical dilemmas with respect to disease prognosis and treatment choices, saying in personalized medicine. Due to insufficient evidence for PGx, esp. on a specific case basis, as well as additional cost of time and money for PGx, people often wonder who on earth needs the PGx test and what exactly does the PGx tell us? Relevant issues are sorted out as follows.

12.2.1.1 Lack of Association

The first ethical challenge of PGx towards personalized medicine is how to fully confirm the evidence and make clear association between genetics and drug response. Only with sufficient association could it be implemented into clinical

practice, and acquire public acceptance without doubts. Up to date, Approx. one-third of Food and Drug Administration (FDA)-approved drugs with PGx information in drug labels and half of the European labels posted on PharmGKB website contain recommendations on genetic testing [11]. Research proved it was possible to use relative P450 enzyme, i.e. CYP2C19 genotype-dependent hepatic activity and variability to predict pharmacokinetic variability and possible drug–drug interaction [12]. But in addition to genetic variant, adherence, age, race, comorbidities and drug–drug interactions, etc. also contribute to variability in drug response. Moreover, gene variants are sometimes pleiotropic, and are associated with more than one disease or drug response. Only with convincing and direct associations between gene and drug response, could it be forced the inclusion of PGx into clinical practice, which aims to avoid malpractice litigation to a potential severe adverse drug effect. Otherwise, foreseeable harms might outweigh anticipated benefits for participants in PGx research, and as well as in clinical practice. Moving forward, studies are needed to show that the risk–benefit ratio of a drug is improved by PGx testing. Nowadays, most PGx tests are not covered by premium, or rather, basic medicare, which blocks PGx in some extents. As a coin has two sides, so is this issue. Healthcare providers may be willing to allocate resources for PGx tests or personalized drugs if there is evidence of the benefit in terms of savings in health expenditure, in the long term, for treating the rare conditions.

12.2.1.2 Ethnic Differences

Genotype and phenotype are not directly correlated with each other, often due to factors that can affect the regulation or activity of the polymorphic gene, or other non-genetic influences on phenotype, including ethnicity, disease, age, diet, use of alcohol, and co-administered drugs, etc. The ethnicity means a lot of the environmental and habitual factors. There are numerous examples demonstrating the prevalence of genetic polymorphisms varying between ethnic groups. As reported, more than 25 single-nucleotide polymorphisms in 19 genes have been investigated in association with AIWG in Chinese patients over the past few decades. HTR2C rs3813929 is the most frequently studied single-nucleotide polymorphism, and it seems to be the most strongly associated with AIWG in the Chinese population [13]. A review of 120 studies conducted on 51,747 Brazilian healthy volunteers reported the differences in allele frequencies between color categories that persist despite the homogenizing effect of >500 years of admixture. Among clinically relevant variants: CYP2C9∗2 (null), CYP3A5∗3 (defective), SLCO1B1-rs4149056 (C), and VKORC1-rs9923231(A) are more frequent in Whites than in Blacks. Brazilian Native Americans show lower frequencies of CYP2C9∗2, CYP2C19∗17 (increased activity), and higher of SLCO1B1-rs4149056(C) than other Brazilian populations [14]. However, most PGx studies in Chinese subjects are conducted in individual hospitals or research centers. Results of these single hospital or center studies are sometimes hard to reproduce or even conflict with one another [15]. Anyhow, the human responses of a drug differ between ethnics and colors, resulting in

more complexity in the PGx tests and their interpretations, and issues relevant rise consequently.

12.2.1.3 Quality of Informed Consent

The concept of informed consent is absolutely a cornerstone in modern medical research ethics and a part of most national legislation concerning medical research. As interpreted in International Conference of Harmonization (ICH) and Good Clinical Practice (GCP), i.e. ICH-GCP, informed consent is a process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. And it is documented by means of a written, signed, and dated informed consent form (ICF). Generally speaking, the information disclosed to subjects in ICF must include in a comprehensible way of the research procedure, its purpose, anticipated benefits and harms, alternative treatments (if available), an invitation to ask any questions, and a statement that the subject may withdraw from the study at any time. A qualified informed consent must be given by somebody who is psychologically capable of consenting, and the text in the consent form shall include possible implications for the participant concerning issues, including but not limited to privacy, confidentiality, re-contacting, and in particular, the storage of samples and long-term dispositions, as much as possible. Regarding genetic testing, there appears to be no standard at the national or international level concerning how to consent individuals. In a survey of oncology services providers, many cancer patients were unable to adequately comprehend the purposes and complexities of PGx testing [16, 17]. In the PGx field, the evidence base of genetic testing should be informed by the pharmacologic characteristics of the drug and the characteristics of the outliers. In China, informed consent is required by the State Council, and collecting, storing, using, and/or providing genetic resources should be done after obtaining the consent from the providers in advance, and the legal rights of providers should be protected.

However, it should be noted that signing informed consent by family members other than the patient, especially when the individual has severe disease, is a common phenomenon in many countries. In China, white liars of hiding the true disease or its conditions are still prevailing in some regions, particularly in areas with low education levels. Despite the original kind-hearted willingness, an informed consent is never done sufficiently in this scenario to the participant, or even to the family members. It might deteriorate the relationship between doctor and patient to give insufficient information of a consent. As a result, a family consent is worth to be taken into account in medical practice. Considering the possible common genetic features of family members, ICF of PGx is more necessary to view the broad consent. There is some disagreement as to whether broad consent can ever be equated with genuine informed consent [18, 19], although most ethicists are in favor of its use and agree that this is a decision ensuring the participant's autonomy.

12.2.1.4 Vulnerable Groups

Consent of participation is only considered valid when responding to three elements: information, comprehension, and voluntariness [20]. Certain groups of people such as children may not have the capacity to make free and informed decisions. In the case of children or adolescents, who should be capable of giving assent for a PGx test, the issue is very complex, due to their capacities to understand the implication of the matter. A method to protect children against the possible negative effects of participation in research is to use both consent and assent. In this context, pediatricians have a central role to ensure the strict respect of regulatory and ethical requirements as well as good clinical practices [21]. In some countries, a written ICF for both parents is mandatory regarding PGx test, like in the USA, but not in Asian countries, like China, Japan, India, etc. [20] Sometimes, the genetic information could have an impact on a child's clinical care and the parents may not be equipped to understand the impact on the overall health of their child. Some elders and disabled people are also considered to be vulnerable, since they may not understand the actual purpose, method, and long-term perspectives of PGx when being consented. Vulnerable persons also include those with diminished competence and/or decision-making capacity due to medical conditions. Understanding PGx tests is even more difficult when the patient has a lower educational level. On the other hand, the trial participant may affirm that they do not want to be re-contacted in future and are prepared to give authorization for the researchers to use their material anyway. From an ethical perspective, such a position would also be a voluntary decision and thus guarantees the principle of autonomy.

People in low-resource setting counties or areas are another population in vulnerable groups. Although some genomics initiatives have begun to emerge in developing countries such as Mexico, Gambia, and China, for the majority of countries and areas in the developing world, including north and west area of China, PGx seems to be far out of reach. PGx testing might not be cost-effective in these area where basic healthcare is limited and appropriate drugs are not always available. Further research is needed to address this issue.

12.2.1.5 Human Rights vs Responsibility

The Declaration of Helsinki (DoH) is the World Medical Association's (WMA) best-known policy statement. Since 2016, the Declaration of Taipei on Ethical Considerations regarding Health Databases and Biobanks has complemented the DoH. Biological material refers to a sample obtained from an individual human being, living or deceased, which can provide biological information, including genetic information, about that individual. Health Databases and Biobanks are both collections on individuals and population, and both give rise to the similar concerns about dignity, autonomy, privacy, confidentiality, and discrimination.

Keeping genetic information in complete anonymity is neither technically feasible, nor desirable [22]. Several Biobanks currently use a broad, general consent, since future use of such PGx data may not be known at the time of consent. In the USA, The Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations include federal standards applicable to all the US facilities or sites that test human specimens for health assessment or to diagnose, prevent, or treat disease. CLIA does not regulate whether a laboratory must document informed consent prior to performing the requested tests, but the recommendations emphasize that all testing decisions and subsequent patient treatment be based on properly informed decision making. Additionally, some states require informed consent be obtained before genetic testing occurs. However, in the current genomic era, participants may be unaware of the true extent of the researcher's promise of privacy, confidentiality, and anonymity when giving their consent. As a result of extensive data sharing, the emergence of large-scale research platforms, and the unique fingerprint nature of DNA, the significance of those promises should be reconsidered.

In addition, human rights in PGx area include not only the right of future procedure regarding his sample with genetic information, but also the rights of NOT to know the result! An important issue that needs to be considered for whole genome or exome sequencing in the newborn phase is that the cost of sequencing the whole genome, will soon be practically, not more expensive than sequencing targeted genes. However, this brings ethical issues such as protection of the future autonomy of the screened infant, the right not to know, and the issues around incidental findings. Should ancillary findings be returned to parents when children have been the subject in research? Do parents have an ethical duty to disclose the results to their child? Do researchers have an ethical duty to communicate research results? Communicating PGx or genomic test results to children raises some questions such as who should receive the results (e.g., the child, his or her legal representative, his or her doctor) and in what capacity.

Stigmatization is defined as "a social process that begins with distinguishing and labelling some feature of a person such as occupation, disease, or skin color, or from his geno-dividing". Should participants receive feedback on their individual research results? It has been argued that validated, verified, and clinically useful research data should be returned to the participant (if requested by the participant), based on the classical biomedical ethical principles of respect for the person, beneficence, and justice. An individual may experience stigmatization from family, friends, and coworkers on knowledge that a specific disease will not respond to therapy or if one is identified as a "poor metabolizer" of a specific medication. This may cause the individual to feel lonely, isolated, hopeless, and depressed.

In addition, question arises as to how to include informed decision making for re-contact in the consent. How does one know in advance what information a participant would want to be provided with and what not? Especially concerning clinically relevant data that may emerge during the study but that do not address the main research question (secondary information), this is a complex matter. As declared in Helsinki, the genetic information is personal collections. However, to know the geno-information is for the comment good, not the specific person himself

when PGx tests are required, e.g. in clinical studies. So, the individual willing may conflict with the population needs, needs in scientific view. Does a part of a population have the responsible to join the seeking, or studying on ethnic PGx?

12.2.2 Legal Issues

As we discussed above, PGx tests involve many parties, from subject (patient or healthy participant) and investigators to all companies, like pharmaceutical companies, analytical corporations, insurance providers, etc. The perspectives of each group will vary, but as all have a vested interest in a successful outcome, and they depend on each another, there is a willingness to harmonize effective practice to move forward. However, it is not enough to guarantee ethical issues are fully respected, there is a requirement for good governance, with a variety of regulatory instruments applicable at various stages of research. As the PGx is developing fast, legislations are usually lagging behind, and legal issues are raised consequently. Here we discussed the issues from two major aspects, one is the process of PGx test, both in clinical research and practice; and the other is all views towards PGx data, involving the information implied.

12.2.2.1 Process of PGx Test

PGx testing would include procedures from acquiring individual's consent, collecting bio-samples, carrying out testing to reporting results. Acquiring ICF or exemption of ICF is a first issue among considerable debates about the quality, quantity, and type of evidence needed to change clinical practice by introducing PGx testing, though it is not an emergency [23, 24]. How to acquire ICF regarding PGx sufficiently is, currently lack of legislation, as we discussed before. Considering that the DNA collected will not just include the known genetic polymorphisms obtained in a research study but may also include information about the individual's entire genome. As a result, these DNA samples could provide invaluable information about his or her extended family, which could be entirely unrelated to the original study. In this respect, informed consent is a legal issue, involving the whole family members. It is noted that in many clinical trials, residuals of bio-samples like blood or plasma are used, and most of these trials have the exemption of ICF as reviewed by IRB in an institution. In China, most clinical trials of gene-kits are exempted of ICF, as long as they use residual samples storing in laboratory after previous normal medical test, which often aims at certain gene information and more would be revealed by the follow-up gene-kit trials. Do the sample providers have the right to know more of their geno-info? Do they need to assent the reuse of their bio-samples, though the follow-up trials are not foreseeable in advance? From a subject-donor perspective, his or her biological sample is also considered as a property right, so denying such a right is unfair. Anyhow, it is often difficult to contact the providers of ready-to-dump

bio-samples in hospitals, and the re-contact is not expected, or rather welcomed by the patients themselves even if it is possible.

In most countries, the principle that "a clinical trial cannot be carried out without an affirmative vote of an ethics committee" is stated, regardless of written or oral consent or even exemption, but it is often less restricted for PGx tests in clinical practice on purpose of disease diagnosis or drug prescription and monitoring. At times, biological specimens are aliquoted whereby material is used, so the ownership of data matters. Large databases and bio-banks have emerged to store biological specimens and genetic information. In Netherlands, databases are used by the majority of general practitioners and community and hospital pharmacists; while in China, there are some similar Biobanks, e.g. Chinese National Immortalized Cell Bank, established in 2002. It has preserved human LCLs from different ethnic groups in China. And updated to the end of 2016, 1982 types of cell-lines and 9902 lots of DNA and serum samples in total were preserved, from 39 ethnics and regions [25]. More often in China, most samples after medical treatment are temporally stored in hospitals as residuals, which might be kept for years and reused in some clinical trials, especially for new gene-kits trials. Clinical practice guidelines are rare, and labeling content is limited. Up to date, no files have been archived for the informed consent of after-treatment residuals in China. But in the USA, some lawsuits were recorded [26]. Aside from public collections, pharmaceutical companies have maintained their own private collections of human biospecimens from clinical trials for many years, but these have largely been unavailable to external users, except some companies, such as 23andMe using their databases to engage in research programs.

Regarding the PGx test experiments, they are performed in certificated laboratory, either located in a dependent institution, or affiliated in a hospital. However, the test procedures are sometimes debatable, not in technique, but in property right. Approximately 20% of all human genes are under US patents, and patent system is protected as inventions. In 2009, a lawsuit was filed by the American Civil Liberties Union (ACLU) and others against Myriad Genetics for their US patent on the human genes BRCA1 and BRCA2, which are two mutations associated with an increased risk of breast and ovarian cancers. Myriad Genetics owned at least seven patents directed at BRCA1 and BRCA2 at that time, but the plaintiffs argued that the BRCA genes were products of nature and were thus outside the realm of patent protection. In July 2011, the Federal Circuit ruled in favor of Myriad Genetics and declared that BRCA1 and BRCA2 genes were patent-eligible under Section 101 of the US Patent Act.

12.2.2.2 Protection of Personal Data

As PGx testing would unveil an individual's genetic information, and to his or her extended family as well, issues related to discrimination or stigmatization are mostly discussed when individual's geno-information is accessed by employers and insurers. We should take seriously weigh and address public fears concerning the

misuse or inappropriate disclosure of genetic information by third parties. This fear might discourage people from participating in PGx research. Knowledge of genetic and/or PGx information may be used to deny, limit, or cancel health insurance. This practice is caused mainly by ignorance, confusion, and misunderstanding, but is also the result of a lack of clear legal definitions of genetic data and tests, e.g. the PGx tests. There should be limited access to such geno-information. Insurers would be able to use PGx information for drug formulary management, but should be prohibited from using the same information in determining copayments or premiums, or negotiating contracts. Additional concerns regarding genetic discrimination include employers using such information to only employ or retain individuals who do not have the genetic disorder or genetic polymorphism, limiting access to social services, and in the delivery of health care.

Belgium was the first European country to introduce genetic nondiscrimination legislation in 1990, and many countries followed. In addition, in 2008, after several years of pending in Congress, the USA adopted the Genetic Information Nondiscrimination Act (GINA), which promised to provide extensive protection against the misuse of genetic information by employers and insurers, and was intended to encourage US citizens to participate in clinical research and genetic testing. However, there is an ongoing debate on how well these antidiscrimination laws actually protect the privacy and confidentiality of individuals.

PGx data may not be known at the time of consent. Taking residual bio-samples into account, future researches are not predicted in advance, so that the future disclosure of PGx information is inevitable. Data sharing is considered to be an essential part of the current genomic research process, and can be regarded as a form of health information altruism, in particular, for the unexpected disclosure or those without consent. In China, Ministry of Science and Technology (MOST) is in charge of human genetic resource management (HGRM). The most recent rules of HGRM stipulated that the follow-up handling method and time should be clarified, involving the image data if it contains some genomic information [9]. In the USA, Health Insurance Portability and Accountability Act (HIPAA) and private sector self-regulation exist. The HIPAA Privacy Rule provides federal protections for personal health information held by covered entities. However, uncertainty remains in the capacity and ability of current systems to ensure privacy, security, and confidentiality. Nonetheless, there are contrasting federal and state law privacy standards. In one scenario, if a provider orders a genetic test from one state and the results of the test are made available electronically to another out-of-state provider, clarification is warranted as to which state privacy laws are followed. In China, it seems that the public have not drawn high attention to future disclosure of their PGx information. It may be partially attributed to the fact that the physician's records in different hospitals are not connected or shared in most provinces nowadays, and neither the third party nor even the individuals would get much useful information.

12.2.3 Social Issues

Apart from the ethical and legal issues as mentioned above, there are many other issues rising from daily life which have relevance to PGx, rather than the participants or subjects, but the general public opinions on PGx, involving parties' function and responsibilities except for traditional doctors and patients, but people in other circulations, who are included in some direct-to-consumer commercial tests for specific purpose. Some of these issues are discussed representatively as follows.

12.2.3.1 Acceptance of PGx

Usually, PGx tests are prescribed by physicians to screen the genetic characteristics towards drug response, or to potentiality of certain genetic diseases. A distinction was proposed for screening test which might be useful for PGx test as well, since screening purse of PGx test is more and more popular. Screening is divided as follows: (1) high-quality responsible screening leading to health gains, (2) screening that does not convincingly lead to health gains and is not harmful, and (3) risky or unsound screening. Currently, the PGx tests are often performed in laboratory, which seems to have less authoritativeness in medical views, resulting in less acceptance by the public. In addition, there are some technical challenges, like (1) reproducibility stems from tumor sampling, (2) confidence of the assay, when sample size is generally very small relative to the number of array features tested, (3) correction for multiple testing when isolating differentially expressed genes or associating SNP variants with a particular phenotype, (4) logistical challenges, etc. On the other hand, PGx tests are mostly non-regular tests in healthcare providers, saying hospitals and community healthcare centers, probably due to lack of association of genetics to diseases, and relatively low acknowledgement of public.

12.2.3.2 Affordability of PGx

In many countries, the price of PGx tests is noticeable, which leads to less acceptance and more hesitation of taking the PGx tests. For example, a specific disease or drug related gene-test usually costs thousands to over 10,000 Chinese yuan; while regular PGx tests for target drug monitoring are about a 100 yuan per time, and patients are in need of repeated tests for a long time. Nowadays, the monthly disposable income of a person in 2018 is $<$3000 in China. Even in the USA, the cost and benefits of PGx are still debating. Healthcare providers may be willing to allocate resources for PGx tests or personalized drugs if there is evidence of the benefit in terms of savings in health expenditure, in the long term, for treating the rare conditions. However, in the USA or European countries, not all genomic tests

are covered by insurance and this deters patients from obtaining the test and moreover the availability of insurance cover is not uniform, i.e. varying from state to state in the USA. In China, most PGx tests are not included in the governmental medical care project, despite that some PGx test are more and more convincing, and prescribed by physicians quite often. In other words, general affordability of PGx test conflicts with its necessity in some extent. And as well, issue pertains to payer coverage and reimbursement for companion diagnostics and prognostic tests.

12.2.3.3 Credibility of Commercial PGx Tests

The definitions of genetic testing used by a wide range of organizations and entities (e.g., professional genetics organizations, insurance companies, pharmaceutical companies, and law firms) are extremely varied and—especially in the legislation area—often very inconsistent [27]. Issues affecting the other stakeholders will also be pointed out. An increasing number of companies are starting to offer health-related genetic testing services directly to the public. The direct-to-consumer (DTC) PGx tests offered may predict response to medication, such as HER2/neu testing prior to prescribing trastuzumab and CYP450 gene testing related to liver metabolism of many commonly prescribed drugs. Nevertheless, critics have raised concerns regarding the limited access to genetic counseling, uncertain laboratory quality of the tests, the accuracy and adequacy of the information provided by companies, the protection of privacy, and the risk that consumers may be misled by false or misleading claims. Regulation of DTC testing is under debate. In China, over 100 genes are tested regularly, either in hospitals as TDM (Therapeutic Drug Monitoring) for patients, or in some testing company for consumers.

12.2.3.4 Public Education of PGx

More education of PGx is a public consensus, since the education level influences the understanding of an informed consent, the acceptance of the test, and most important, the appropriate application and interpretation of PGx result. Issues rise from conflicting acknowledgment of PGx by different parties, e.g. patients vs doctors in particular. Low education level will bring barriers of the pipeline of PGx in pharmacy. In current tests for licensed pharmacists, certified doctors, and even nurse practitioners, PGx is included in China. But integrating PGx into pharmacy education and practice is not enough. Probably, incorporating PGx into undergraduate education is expected, and popularization of the PGx concept to the public could be helpful to resolve and reduce relevant issues. Regarding the education program, not only government but also other stakeholders ought to take their responsibilities, including commercial companies in the pharmaceutical industry,

who have a duty to society to invest in research and development of new drugs to provide efficacious and safe drugs through properly conducted clinical trials. It is believed to be of long-term benefit altruistically to the general public.

12.3 Rules and Regulations in Pharmacogenomics

The first official document on pharmacogenomics is proposed by US Food and Drug Administration (FDA) in 2005 [28]. So far, several guidelines or regulations have been proposed. Some important pharmacogenomics official documents are shown in Fig. 12.1. As most of FDA and EMA rules and regulations have been reported thoroughly in literatures, they are briefly discussed thereinafter, together with some important documents proposed by China government.

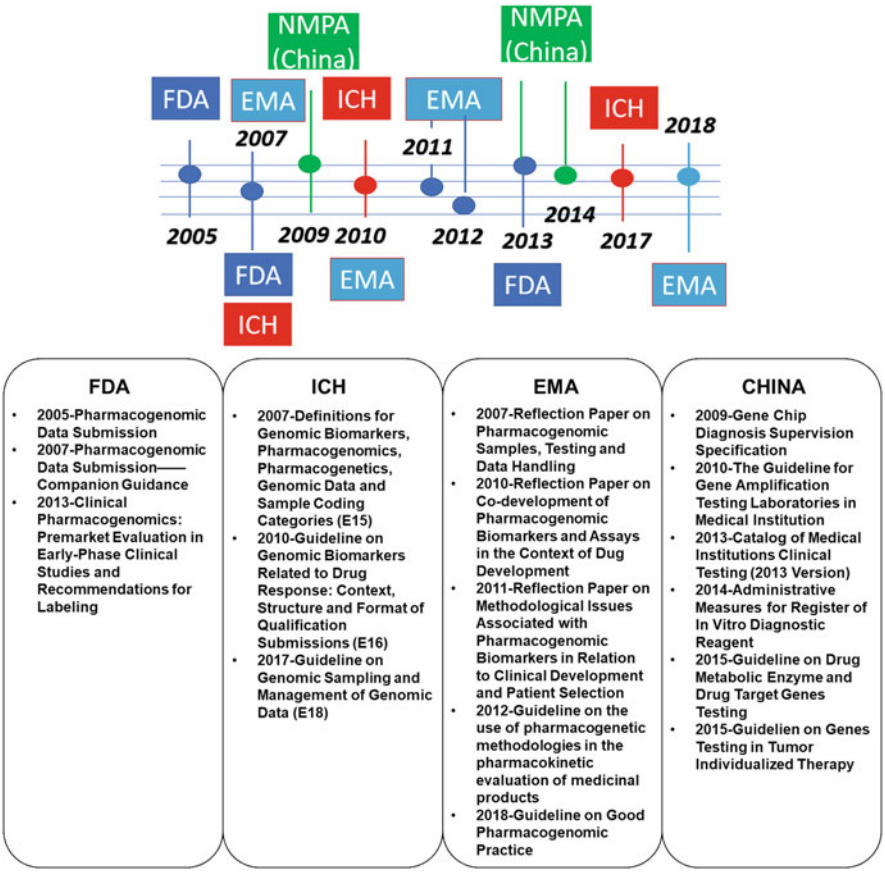

Fig. 12.1 Important pharmacogenomics official documents

12.3.1 Clinical Researches

Gene mutation testing demands high quality genomic DNA and sample quality and amount can influence the accuracy and reliability of the generated data [29, 30]. Therefore, quality assurance of collection, transport, storage, and handling of samples are critical in both clinical researches and practice. There are several guidelines and reflection papers addressed quality assurance, including collection, transport, storage, and handling of samples [29–32]. The content of discussion below is based on the latest release. It is advisable to minimize the pre-analytical variations throughout the workflow. Some retrospective pharmacogenomic related studies are often performed on stored samples. Thus, establishment of dedicated pharmacogenomic sample repositories that adopt strict supervision standards should take into consideration. The testing procedures require proper validation, that may need conduct in different countries or subject to using nationally accepted procedures.

Privacy is a noteworthy issue in both clinical study and practice. In clinical study, coding of genomic samples and data is a powerful method to protect privacy. Coded data and samples are classified into identified, coded, anonymized, and anonymous status [33]. Coded data and samples can be subdivided into single coded and double coded. In clinical research, samples and data should be single coded at least. However, with the increasing availability of genomic information and analysis methods, previous defined coding ways may not be applicable in nowadays. It is pointed out that anonymization, as defined in ICH E15 [33], in which coding keys have been deleted and made re-identifying subject impossible now becomes possible [30].

Informed consent is a controversial issue involved in both ethics and legal. It is an important part of Good Clinical Practices (GCP) considerations. The principles of consent for pharmacogenomic research are similar with that in GCP. In ICH E18 [30], some details of consent for pharmacogenomic research are discussed. To obtain suitable informed consent, details of collecting samples, procedures in collection, the position on returning genomic data, and the genetic counseling, if it is possible, all should be described in consent. Also, participants who can only be enrolled in the study with the consent of the subjects' legal representatives or guardians should also be considered.

In China, the official document on use of pharmacogenomics in clinical trial is rare. Chinese government not allowed researchers to perform testing like gene testing that is not related to the protocol approved by the Ethics Committee [34]. If the remaining specimens are saved or may be used in the future when clinical trial is over, the subject should sign the informed consent that would explain the time of sample preservation and the confidentiality of the data. To safeguard human genetic resource in China, Chinese issued relevant regulations in 2019. In this regulation, Chinese government clearly stipulates that any organization hopes to use human genetic resources in China to conduct clinical trials needs to meet the rules and regulations of clinical application management [9]. Besides, when foreign

organizations need to conduct international incorporated clinical trials to get marketing authorization of drug or medical device in China, there is no need to get approval when genetic materials do not involve departure. However, before clinical trials start, cooperated partners shall submit the information of the type, quantity and use of genetic materials that would be used to the Ministry of Science and Technology.

12.3.2 Clinical Practice

In 2009, ministry of health of China issued a supervision specification that lists the requirements for medical institutions, medical staffs, and technology supervision in the use of gene chip [35]. For example, gene chip diagnosis should be conducted in the laboratory certificated by the national center for clinical laboratories. Medical staffs should receive systemic training of gene chip technology to get their qualification. Also, medical institutions and staffs should be regularly assessed their capability of using gene chip technology for clinical application. All uses of gene chip should be approved and supervised by the hospital Ethics Committee. Only the reagents and devices that approved by CFDA (China Food and Drug Administration, now it has been renamed as National Medical Products Administration, NMPA) can be used. In 2017, the National Health and Family Planning Commission issued another related regulation. The content of it is similar with previously discussed [36].

According to administrative measures that were issued in 2010, the certification of laboratory is executed by provincial center for clinical laboratories or other institutions designated by provincial health administration departments [37]. Testing items must be registered and approved by provincial health administration departments before conducting. Laboratory staffs should be qualified by the designated institution of health administration departments above the provincial level before work. Regular work of laboratories should refer to the another regulation issued in the same year [38]. If the gene testing is for research purpose, laboratories are not allowed to produce the corresponding report and charge any fees.

The ministry of health stipulated that all types of medical institutions at all levels should not carry out any testing items outside their issued catalog. In the catalog, some major genes related to individualized therapy were included [39]. The details of these genes are listed in Table 12.1. However, the items listed in the catalog (version 2013) are still too less to meet the needs in clinics. In 2016, ministry of health stipulated that unlisted clinical testing items which have clear clinical significance, good specificity, good sensitivity, reasonable price, and reasonable benefit are encouraged to timely certify.

In 2015, to promote individualized therapy, National Health and Family Planning Commission proposed two relevant guidelines involved gene testing [40, 41]. It means that quality assurance were addressed in the both guidelines. The content of quality assurance in them is similar with previous discussed. It contains sample collection, sample store, staff training, testing methodology, and results analysis.

Table 12.1 List of individualized therapy related genes in *Catalog of Medical Institutions Clinical Testing (2013 version)*

Molecular biology testing on tumor therapy	Molecular biology testing on medication guide
Qualitative testing of hematonosis related genes	Chemotherapy medication guide related gene testing
Quantitative testing of hematonosis related genes	CYP2C19 gene polymorphism testing
Leukemia fusion gene testing	CYP2C9 and VKORC1 gene polymorphism testing
Human EML4-ALK fusion gene testing	MTHFR (C677T) gene testing
Prediction of tumor therapy prognosis based on gene expression level	CYP2D6∗10, CYP2C9∗3, ADRB1(1165G>C), AGTR1(1166A>C), ACE(I/D) testing
Prediction of antitumor drug sensitivity based on gene expression level	Hepatitis B virus drug resistance gene testing
Human PIK3CA gene mutation testing	Mycobacterium tuberculosis drug resistance gene testing
Human EGFR gene mutation testing	Vancomycin resistance gene testing
HER-2 gene amplification testing	Methicillin-resistant staphylococcus drug resistance gene testing
Human K-ras gene mutation testing	Pathogens medication guide gene testing
Human B-raf gene V600E mutation testing	
Human P53 gene mutation testing	
Retinoblastoma RB1 gene mutation testing	
Familial breast cancer gene mutation testing	
Multiple endocrine neoplasia RET gene mutation testing	
Hereditary nonpolyposis colorectal cancer gene mutation testing	
Hereditary colorectal cancer microsatellite instability testing	

The details are discussed briefly here. For example, any testing that involves collecting gene data should get the informed consent from patients before collection. The informed consent form should include the potential risk, aim, clinical significance, limitation of testing, and the preservation of sample in case of re-examination. To protect the privacy of patients, all parts involve diagnosis and reporting should kept confidential, and without authorization no information should be disclosed. The reports of diagnosis should be only sent to the applicants and patients. Some ways could ensure the confidentiality. For example, patients need show valid credentials to receive reports. If the reports could be inquired in self-service machines, patients' own medical card needs to be swiped. However, if patients do require or consent information sharing after informing the related regulations, the reports could be sent to others. Sample store is another issue worth discussing. After reporting,

remaining samples should be preserved in the long term or at least, preserved in the whole time of patient therapy. If physicians have any doubt about the report, the feedback should be given to laboratory as soon as possible.

The choose of testing methods was also discussed [41]. For example, Sanger sequencing, one of the most classical DNA sequencing methods, is regarded as the gold standard of genotyping. However, due to its lack of sensitivity, the content and proportion of tumor cells in sample should be high so it is not applicable in biopsy or cytological sample. The choose of tumor gene testing method does not have unambiguous suggestions. It should be considered case by case. Although gold standard methods or methods with good precision and sensitivity are preferred, sometimes priority may be given to other factors like the requirement of sample content. Here is an example. The choose of quantitative real-time PCR needs more than 1 μg total RNA. However, when the size of tumor tissue is too small, the quantity of extracted RNA may not meet the need. In that situation, other methods may take into consideration.

Except gene testing in certificated laboratory, some gene testing kits were developed for easy use. According to government regulation, gene testing kits were classified into in vitro diagnostic reagents [42]. The definition of in vitro diagnostic reagents here is reagents that applied for prediction, prophylaxis, diagnosis, therapy monitoring, prognosis observation, and health status evaluation in vitro and also can be used alone or combine with instruments, appliances, equipment, or systems. However, diagnosis reagents that used in blood source screening or labeled by radionuclide are not applicable for this administrative measure. According to their potential risk, from low to high level, in vitro diagnosis reagents are classified into three types. All reagents related to gene testing are classified into type 3, which is with the highest potential risk. Clinical trial should be conducted to apply for their registration. And an amendment that issued in 2017 gives CFDA the right to adjust the classification rules [43].

In practice, drug labeling also plays an important role in clinical application. In China, there is no rules or regulations involved in pharmacogenomic information in drug labeling. However, such contents are mentioned in FDA proposed guidelines [44]. In general, when informing about the impact of genotype on phenotype is useful, pharmacogenomic information should be include in labeling. Sometimes, available genomic testing methods and the necessity of such testing should be indicated. Except basic introduction of genomic variants, the effect of genotype on PK or PD and dosing and patient selection recommendations based on genotype can also be described.

12.4 Conclusions

Along with the rapid developments of genetic technology, more clinical evidence has been built to correlate genetics with medications, esp. to their effects. In other words, the clinical implementation of PGx testing for most drugs has already been

translated from bench to bedside, saying more and more accepted. At the meantime, the issues and conflicts related to PGx from the ethical point of views have drawn more and more attentions, from specific patient participants and their relatives to general public, including potential consumers out of clinics. However, the process of collecting biological samples, storage of the bio-specimen, and disclosure of individuals' private geno-information are still the major concerns, which are in debating of their standardized procedure with highest public acceptance for the benefits of most general population and ethnics. A practical "sufficient consent" has yet no clear definition in any rules, but is in research on uncertainty picture of processing and sharing genetic information worldwide, both national and community level. For many developing countries, e.g. China, the prevalence of PGx testing is soaring in big hospitals, in versus of relatively lagging off legislation by the government, which is pacing up and taking into account of their own situation and characteristics.

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Chapter 13
Cost-Effectiveness of Pharmacogenomics-Guided Drug Therapy

Zhijia Tang and Weimin Cai

Abstract In healthcare settings, whether the added benefit of a new medical intervention is worth its added cost has long been a sophisticated question for clinicians, researchers, and decision makers. All healthcare professionals are expected to understand the philosophy of pharmacoeconomics in order to ensure the appropriate use of medical resources. Using pharmacogenomics-guided treatment, unreasonable medical expenses are avoided as dose being optimized, drug efficacy being enhanced, and side effects being reduced with the aim of personalizing treatment. This chapter identifies key aspects of pharmacoeconomics, including different elements and different types of pharmacoeconomic analyses, and discusses the situation why pharmacoeconomics is essential when utilizing pharmacogenomics in clinical practice.

Keywords Pharmacoeconomics · Pharmacogenomics · Pharmacogenetics · Personalized medicine

13.1 Overview

The USA spent about $2.7 trillion on health care in 2010, for an average of about $8000 per person, or about 17% of the gross domestic product (GDP). About 12% (over $900 per person) of health care expenditures were for medications [1]. Drug cost has become an important factor in healthcare decision-making besides drug safety and effectiveness and for promoting drug accessibility and rational drug use. However, the continued increase in costs limit patient access to treatment when the patients bear a heavy financial burden to buy drugs. This contradiction between a rapidly rising demand for healthcare services including medications and limited resources led to the burgeoning of pharmacoeconomics. Some clinicians believe

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_13

that the so-called cost-effective drugs or treatment strategies may save enough to offset their cost by decreasing the need for more expensive treatment such as surgery or hospitalization or the probability of morbidity and mortality. Pharmacoeconomics combines these objectives by using various analysis methods to measure healthcare outcomes weighed by the costs and benefits associated with drugs or treatment strategies. In today's healthcare settings, individualized treatment guided by pharmacogenomics predicting efficacy and toxicity of drugs is a valuable application field for pharmacoeconomics in reducing healthcare costs while maintaining efficacy.

13.1.1 Definition of Pharmacoeconomics

Pharmacoeconomics is often regarded as a branch of health economics. However, pharmacoeconomics is a multidisciplinary field as the basic tenets of its conceptual framework overlap with both health economics and pharmacy-related clinical or humanistic outcomes research [1]. The necessity of development of pharmacoeconomics was shown when total health expenditures increased substantially worldwide since the 1950s. Healthcare professionals encountered challenges achieving maximum improvement in healthcare outcomes amid limited healthcare resources. To solve this situation, the US Congressional Office of Technology Assessment (OTA) began to analyze the potential application of cost-benefit analysis and cost-effectiveness analysis in the field of healthcare from the economic dimension in the USA in 1979.

The term *pharmacoeconomics* first appeared in 1986 in an article titled *Postmarketing Drug Research and Development* by Raymond Townsend [2]. *PharmacoEconomics* was established in the USA in 1989 and published in 1992 as the first journal in the field of pharmacoeconomics. Later, Jesse Lyle Bootman, Raymond Townsend, and William McGhan edited a book, *Principle of Pharmacoeconomics*, which was the first textbook in this discipline. The International Society for Pharmacoeconomics and Outcomes Research (ISPOR) was founded in 1995 with the goal of improving decision-making for health globally based on scientific research excellence. ISPOR now has more than 20,000 individual and chapter members from over 110 countries worldwide.

Pharmacoeconomics can be defined as the process of identifying, measuring, and comparing the costs, risks, and benefits of programs, services, or therapies and determining which alternative produces the best health outcome for the resource invested [3]. Similar to traditional economics, pharmacoeconomics assesses the choices that a decision maker selects and the cascading costs and outcomes associated with that choice [4]. This information serves to guide optimal healthcare resource allocation in a standardized and evidence-based manner. More specifically, pharmacoeconomic studies can assist clinicians in choosing the most cost-effective treatment options and help decision-making in the financing and management of pharmaceutical products in the health care system or national health insurance

programs of an individual country [5, 6]. Today, principles of pharmacoeconomics can be applied in various settings including, but not limited to, individual patient decisions, therapeutic guideline determination, government pricing, third party reimbursement, formulary decision-making, and rational drug use.

13.1.2 Costs

Cost is defined as the value of the resources consumed by a program or drug therapy of interest. Costs are frequently classified into four categories: direct medical costs, direct nonmedical costs, indirect costs, and intangible costs [7].

Direct medical costs are the costs incurred during consumption of products or healthcare services, which usually consist of medications, diagnostic tests, clinic visits, hospitalizations, and specialist consultation fees. Direct nonmedical costs also result from treatment or illness but do not involve purchasing medical services [7]. These costs include transportation, special diet, child care services, and other out-of-pocket expenses. Indirect costs are the costs of reduced productivity associated with morbidity and mortality [7] (e.g., lost wages, absence from work, activity impairment). The last category, intangible costs, are those of other nonfinancial outcomes of disease and medical care [7]. These costs are often about patients' feelings such as pain, anxiety, depression, and suffering, which are unquantifiable and hardly measured in the research.

Other types of costs like opportunity and incremental costs are also commonly discussed in pharmacoeconomic studies. Opportunity costs represent the economic benefit forgone when using one therapy instead of the next best alternative therapy [8]. In other words, once a product or service has been chosen, the patient will automatically lose the opportunity to undergo another available treatment. The incremental cost is another main parameter of pharmacoeconomic analysis that represents the additional cost a patient needs to pay off per additional unit of effect taking over.

13.1.3 Outcomes

The healthcare outcomes in pharmacoeconomic studies usually represent the overall consequences from multiple causes and cannot be measured directly [9]. Healthcare outcomes can be categorized into economic, humanistic, and clinical outcomes.

Economic outcomes are the consequences of healthcare services or medical treatment [10] measured with monetary values. Humanistic outcomes are the consequences of disease or treatment on patient functional status or quality of life along several dimensions [10] (e.g., physical function, social function, general health status, patient preferences, and patient satisfaction). Clinical outcomes are the medical events that occur as a result of treatment or illness (e.g., safety and efficacy

end points) [10] which are often addressed in nonmonetary units. Thus, the economic, clinical, and humanistic outcomes (ECHO) model combines multiple attributes of a drug for a pharmacoeconomic evaluation besides safety and effectiveness in traditional pharmaceutical sciences. The cost, quality of life, clinical endpoints, and total healthcare resource utilization are all taken into consideration to evaluate the healthcare outcomes under real-world conditions [11].

However, it should be noted that both costs and outcomes might appear differently when being considered from different perspectives (e.g., patient, provider, payer, or society). Therefore, determining the value of a given pharmaceutical product or service depends heavily on the perspectives addressed and the results must be interpreted with caution.

13.2 Pharmacoeconomic Evaluations

Pharmacoeconomic studies aim at delivering healthcare cost-effectively. There are four basic types of pharmacoeconomic evaluations: cost-effectiveness analysis (CEA), cost-utility analysis (CUA), cost-benefit analysis (CBA), and cost-minimization analysis (CMA). Each method measures costs in monetary units but are different in their measurement of outcomes.

13.2.1 Cost-Effectiveness Analysis (CEA)

CEA is the most widely used method which measures outcomes in natural units (e.g., blood pressure, blood glucose, prothrombin time, life expectancy) in order to compare drugs or treatment alternatives in clinical outcomes ("effectiveness") using the same unit of outcomes. CEA is particularly useful in determining which treatment alternatives represent the best health outcome per dollar spent as the clinical outcomes can be routinely collected in clinical practice and easily interpreted [12, 13]. However, CEA measures only clinical effectiveness and cannot be used to compare alternatives with different unit of outcomes (e.g., blood pressure versus blood glucose). Moreover, CEA cannot measure the opportunity costs of funding a new alternative (allocative efficiency) [14].

13.2.2 Cost-Utility Analysis (CUA)

To address this disadvantage, CUA has been developed. This approach measures cost per unit of health-related quality of life ("utility"), usually expressed as quality-adjusted life years (QALYs) or disability-adjusted life years (DALYs), and allows for comparison of interventions achieving different natural outcomes [15]. CUA is

the most appropriate method to use when comparing life-extending but toxic treatment (e.g., chemotherapy) [16], when morbidity and mortality are both essential for the alternative [13], or when quality of life stands as the most important health outcomes. CUA is able to measure both technical efficiency and allocative efficiency [14]. The main disadvantage of CUA is that the measurement of utility is quite subjective and it is almost impossible to have everyone on the same page during the comparison.

13.2.3 Cost-Benefit Analysis (CBA)

In CBA, both costs and outcomes are measured and converted into monetary terms. As a result, it is now possible to compare alternatives with different unit of outcomes (e.g., blood pressure versus blood glucose) and clinical decision makers can easily make a choice between given alternatives. CBA has the similar drawback as CUA since it may be difficult to reach a consensus on valuing all health outcomes in monetary units. Therefore, unless the benefits of a program or treatment alternative are expressed appropriately in monetary units, CBA should not be employed [17].

13.2.4 Cost-Minimization Analysis (CMA)

CMA differs from other types of pharmacoeconomic methods because it can only be used in comparing alternatives with proven or assumed equivalent outcomes. It is appropriate to employ CMA when comparing two or more therapeutically equivalent agents or alternate dosing regimens of the same agent [17]. CMA does a simple cost saving comparison between alternatives: the less the cost, the better the choice, which may be useful given the increasing number of "me too" products and generic competition in the pharmaceutical marketplace [18]. However, due to its strict application rules, the type of interventions that can be evaluated with CMA is obviously limited.

13.3 Cost-Effectiveness of Pharmacogenomics and Pharmacogenomic Testing-Guided Personalized Medicine

Pharmacogenomic tests are a useful tool to guide treatment and to predict potential drug efficacy and adverse drug reactions (ADRs) before administering medications. Such testing usually focuses on identifying specific biomarkers or genetic mutations, which provide information for diagnostic, prognostic, and predictive purposes

[19]. Considering the clinical and economic burden, researchers in cardiovascular diseases (CVD), psychotic diseases, immunology diseases, and cancers are at the forefront of developing new pharmacogenomics testing as many medications in such fields have significant individual differences due to genetic factors. Gene polymorphisms can affect drug absorption, metabolism, distribution, and excretion, and therefore affect pharmacokinetics and pharmacodynamics. Individual genetic information can be used to predict drug efficacy and the risk of adverse reactions and facilitate a personalized approach to disease management by identifying the safest and most effective treatment options for an individual. Recognizing the importance of pharmacogenomics in identifying responders and non-responders to medications, avoiding adverse events, and optimizing drug dose, the US Food and Drug Administration (FDA) has recently provided guidance on the inclusion of appropriate information on genomic biomarkers in the drug labeling of over 385 drugs [20]. The pharmacogenomic information includes drug exposure and clinical response variability, risk for adverse events, genotype-specific dosing, mechanisms of drug action, polymorphic drug target and disposition genes, and trial design features which indicates specific actions to be taken based on the biomarker information [20]. Despite the empirical evidence supporting the cost-effectiveness of pharmacogenomic testing-guided treatment, some equivocal study results have caused controversy regarding the implementation of pharmacogenomics in healthcare settings. Following are several pharmacoeconomic studies comparing the application of drugs with and without prior genetic testing and investigating the cost-effectiveness of pharmacogenomic testing-guided approaches in clinical practice.

13.3.1 HLA-A∗3101/B∗1502 and Carbamazepine

Our immune system is responsible for immunologically mediated adverse drug reactions (IM-ADRs). During the last decade, strong associations have been found between human leukocyte antigen (HLA) alleles and T cell-mediated drug hypersensitivity syndromes such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Both are characterized by blistering of the skin and mucous membranes. Mortality from these two life-threatening severe cutaneous adverse reactions (SCARs) is close to 30%, with an incidence of 1/10,000 drug exposures in Europe [21–23]. SJS and TEN caused by carbamazepine are related with the presence of the *HLA-A∗3101* allele in European Caucasians or patients of Japanese descent [24] while also with the *HLA-B∗1502* allele in south-east Asian populations, respectively. The latter was initially discovered among Han Chinese in Taiwan [25]. Dong et al. [26] conducted a CUA of *HLA-B∗1502* test in Singapore in 2012. An incremental cost-effectiveness ratio (ICER) was calculated as $37,030 per QALY in Chinese, $7930 per QALY in Malays, and $136,630 per QALY in Indians, which supported cost-effectiveness of *HLA-B∗1502* genotyping in certain Asian populations. Screening of those high-risk groups commencing carbamazepine

is very likely to identify individuals at risk of SJS/TEN. Today, routine screening for *HLA-B∗1502* allele prior to initiation of carbamazepine for epilepsy is recommended for all Asian populations in the USA [27] and for patients of Han Chinese and Thai origin in EU [24] while Australian guidelines still have reservations about the usefulness of screening for the *HLA-B∗1502* haplotype [28]. As to *HLA-A∗3101* allele, a study from Plumpton et al. [29] showed an ICER of £12,808 per QALY for a prior genotyping in the UK. However, the evidence to date has not proven the clinical utility of genotyping for *HLA-A∗3101* as a screening tool. Therefore, the routine screening for *HLA-A∗3101* is not recommended [24].

13.3.2 HLA-B∗5801 and Allopurinol

The prevalence of *HLA-B∗5801* allele is higher in African Americans (3.8%) and Asians (4.2%) compared to Caucasians and Hispanics (<1%) [30]. The association of *HLA-B∗5801* with allopurinol-induced SJS/TEN was first identified among Han Chinese as well. The *HLA-B∗5801* allele was present in 100% (51/51) of allopurinol-induced SCAR patients, compared with 15% (20/135) of tolerant controls [odds ratio, 580.3 (95% confidence interval, 34.4–9780.9); corrected p value $= 4.7 \times 10^{-24}$] and 20% (19/93) of healthy subjects [393.51 (23.23–6665.26); corrected p value $= 8.1 \times 10^{-18}$] [31]. Ke et al. [32] evaluated the cost-effectiveness of genetic screening prior to allopurinol therapy and achieved an ICER of $7508 per QALY in base-case cohort and $7390 per QALY in CKD subgroup in Taiwan. In a study carried out in Korea by Park et al. [33], *HLA-B∗5801* screening strategies increased the probability of continuation of gout treatment without SCARs and reduced the total expected cost. Another study by Cheng et al. [34] showed similar results among Han Chinese. On the other hand, in the study conducted by Chong et al. [35] in Malaysia, *HLA-B∗5801* screening resulted in 0.252 QALYs loss per patient at an additional cost of $322. Jutkowitz et al. [36] founded that testing for *HLA-B∗5801* prior to allopurinol initiation is cost-effective for Asians and African Americans, but not for Caucasians or Hispanics in the USA. The 2012 American College of Rheumatology (ACR) guidelines recommended to screen *HLA-B∗5801* allele prior to the allopurinol initiation for gout in high-risk subpopulations (e.g., Koreans with stage 3 or worse CKD, all Han Chinese and Thai descent irrespective of renal function) while universal screening in whites is not recommended, nor in the USA or EU [24, 37]. The different genotyping recommendations between carbamazepine and allopurinol among different ethnics may be explained by the limited alternatives for treatment of gout [38]. The Singapore Ministry of Health along with the Health Sciences Authority (HSA) jointly issued a Dear Healthcare Professional Letter (DHCPL) in 2016 stating that routine genotyping for *HLA-B∗5801* was not required as standard of care prior to initiation of allopurinol based on current local data in Singapore and it was also not cost-effective from a health systems perspective [39].

13.3.3 CYP2C19 and Clopidogrel

Genetic polymorphism of metabolic enzymes such as CYP2C19 has been identified as one of the key factors responsible for the racial and inter-individual differences in drug metabolism and effect. The frequencies of poor metabolizers (PMs) on CYP2C19 are 15–30% of Asians and 3–6% of Caucasians [40]. The *CYP2C19∗2* and *CYP2C19∗3* alleles account for more than 99% of Asian PMs and 87% of Caucasian PMs. Other new potentially defective alleles such as *CYP2C19∗9*, *CYP2C19∗10*, and *CYP2C19∗12* were identified in African Americans or individuals of African descent [41]. Clopidogrel undergoes CYP2C19-mediated metabolic pathway to an active metabolite and therefore, carriers of defective CYP2C19 alleles will exhibit impaired platelet inhibition by clopidogrel and increased on-treatment platelet aggregation and may need a higher dose of clopidogrel than non-carriers. The high incidence of CYP2C19 allelic variants in Asian population also indicates a potentially greater clinical value. In fall 2019, Mayo Clinic launched a systematic review [42] that 81% (13/16) of the perspective studies included advocated the overall cost-effectiveness of pharmacogenomic testing. Sixty two percent (13/21) of US studies, 75% (3/4) of Canadian studies, 100% (2/2) of Australian studies, 60% (3/6) of the Netherlands studies, and 90% of studies from the remaining other countries of origin were able to conclude that pharmacogenomic testing is cost-effective. Overall, 77% (10/13) of the studies founded pharmacogenetic-guided treatment to be cost-effective in patients with acute coronary syndrome (ACS) with a higher QALY and an ICER ranging from $196 to $70,000 per QALY. Moreover, studies conducted from the healthcare providers, healthcare payers, and healthcare system perspectives had the most positive views regarding the cost-effectiveness (n = 7/8, 88%; n = 14/18, 78%; and n = 7/10, 70%, respectively). The study by Wang et al. [43] supported the cost-effectiveness of *CYP2C19∗2* genotype-guided selection of clopidogrel therapy compared with universal clopidogrel use in Chinese patients with ACS with an ICER of $2560 per QALY. Jiang et al. [44] founded that pharmacogenetic-guided antiplatelet therapy was preferred over universal clopidogrel therapy in Chinese patients with ACS and remained cost-effective in more than 98% of the time. Kim et al. [45] compared the cost-effectiveness of different antiplatelet strategies based on CYP2C19 genotype in the USA patients with ACS, which found that the phenotype-guided approach (clopidogrel + phenotype) was more cost-effective than universal clopidogrel with an ICER of $10,416 per QALY. Another study conducted in the USA by Borse et al. [46] suggested that the incremental cost per major cardiovascular or bleeding event avoided for genotype-guided treatment was $8525 compared with universal clopidogrel.

13.3.4 TPMT/NUDT15 and Azathioprine

Azathioprine (AZA) is an immunosuppressive antimetabolite widely used in rheumatoid arthritis, kidney transplant rejection prophylaxis, and other immunological disorders [47]. Thiopurine S-methyltransferase (TPMT) and nucleotide diphosphatase (NUDT15) are drug metabolizing enzymes responsible of catabolism of thiopurines including AZA. Similar to that of cytochrome P450, the genetic polymorphism of *TPMT/NUDT15* can lead to altered enzyme activity and may induce drug resistance or AZA toxicity due to severe myelosuppression. Recent studies indicated that 10% of patients of European or African ancestry have at least one loss-of-function allele of the *TPMT* gene (heterozygous deficiency) and <1% have *NUDT15* deficiency, while 21% East Asian populations have at least one loss-of-function allele of *NUDT15* [47]. Wang et al. found that *NUDT15* mutation rate was significantly higher in Chinese patients than *TPMT* (20.1% vs 1.4%, $p = 0.000$) [48]. AZA dose should be reduced in patients with *TPMT/NUDT15* deficiency and genotyping or phenotyping is recommended in patients with severe myelosuppression according to the manufacturer [47]. Dubinsky et al. [49] evaluated the cost-effectiveness of different disease management strategies for Crohn's disease in the USA. The *TPMT* screening strategy proved to be most beneficial to reach a response sooner and least costly than standard AZA regimen without a prior predictive test.

A study by Priest et al. [50] conducted in New Zealand founded that phenotype and genotype testing generated net cost savings (vs no testing) of 120,000 and 11,000 New Zealand dollars, respectively, per 1000 patients tested. Marra et al. [51] evaluated the cost-effectiveness of *TPMT* testing in patients with rheumatological conditions in Canada and founded that using a *TPMT* testing prior to AZA treatment can both reduce the cost and ADRs compared to the usual AZA strategy. However, Compagni et al. [52] estimated the net cost of performing *TPMT* testing to avert one case of ADRs (neutropenia) to be €5300 which was a relatively high cost and concluded the solid economic evidences related to the cost-effectiveness of *TPMT* testing are still limited.

13.3.5 KRAS Testing and Cetuximab

Cetuximab is a monoclonal antibody to epidermal growth factor receptor (EGFR) used in metastatic colorectal cancer (mCRC). A mutation in the *KRAS* gene is believed to be involved in resistance to targeted anti-EGFR treatment such as cetuximab and panitumumab [53]. *KRAS* mutations are statistically more frequent in African Americans than non-Hispanic whites [odds ratio, 0.640 (95% confidence interval, 0.5342–0.7666); $p = 0.0001$] [54], while wild type is most common among Asians (66.7%) ($p < 0.001$) [55]. The 2017 National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology strongly recommends

genotyping of tumor tissue (either primary tumor or metastasis) in all patients for *KRAS* at diagnosis of mCRC, and only patients with *KRAS* wild-type tumors should be treated with EGFR inhibitors [56]. Vijayaraghavan et al. [57] estimated the cost-effectiveness of testing for *KRAS* mutations before administering EGFR inhibitor-containing chemotherapy regimens in mCRC in the USA and Germany. Using *KRAS* testing to select patients for EGFR inhibitor therapy saved $7500–$12,400 per patient in the USA and €3900–€9600 per patient in Germany with equivalent clinical outcomes. A Canadian study [53] evaluated the relative cost-effectiveness of testing for *KRAS* genetic mutations, followed by either cetuximab or panitumumab monotherapy, cetuximab-irinotecan combination therapy, or best supportive care (BSC). All strategies with *KRAS* testing were considered cost-effective compared to corresponding strategies of no *KRAS* testing, albeit not equally cost-effective for all treatment options. Shiroiwa et al. [58] performed a cost-effectiveness analysis of *KRAS* testing before administering cetuximab for patients with mCRC in Japan. The ICER of the *KRAS*-testing strategy compared with the no *KRAS*-testing strategy was $230,000 per QALY gained, which was in correspondence with the NCCN guideline recommendations.

13.3.6 HER2 Testing and Trastuzumab

Trastuzumab has been approved in metastatic breast cancer patients who overexpress human epidermal growth factor receptor 2 (HER2). There is a higher frequency of HER2-enriched subtype in Asian patients than in their Western counterparts [59]. Current guidelines from the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) [60] and the NCCN [61] recommend using either immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) at the time of diagnosis or recurrence of all primary breast cancers to determine *HER2* status. Elkin et al. [62] estimated the cost-effectiveness of alternative *HER2* testing and trastuzumab treatment strategies in metastatic breast cancer in the USA. The study obtained an ICER of $125,000 per QALY gained for using initial IHC with FISH confirmation of 2+ and 3+ results, and $145,000 per QALY gained for using FISH alone, compared with no testing and treatment with chemotherapy alone, suggesting cost-effectiveness to use FISH alone or as confirmation of positive IHC results prior to administration of trastuzumab. Another study by Lidgren et al. [63] investigated the cost-effectiveness of *HER2* testing in Sweden. The results indicated that FISH for all patients and trastuzumab in combination with chemotherapy for FISH positive patients is a cost-effective treatment option with an ICER of 561,000 SEK per QALY gained. However, the conclusions from Norum et al. [64] were against the cost-effectiveness of *HER2* testing and trastuzumab treatment for metastatic breast cancer in a Norwegian setting.

As for the adjuvant treatment of *HER2*-positive early breast cancer, Garrison et al. [65] proved the cost-effectiveness of *HER2* testing and trastuzumab in the USA with an ICER of $34,201 per QALY gained. Two studies conducted in Asia by Chen et al.

[66] and Lang et al. [67] suggested that 1-year adjuvant trastuzumab therapy, in combination with chemotherapy, was more cost-effective compared to chemotherapy alone among Chinese and Taiwanese. However, neither study included the cost of *HER2* testing in their model and assumed that patients had already been tested to be *HER2* positive.

13.4 Conclusion

We show in this chapter that pharmacogenomic testing-guided pharmacotherapy could have a positive impact on health-care quality and outcomes. However, the cost of these tests is the essential factor in determining the cost-effectiveness of personalized medicine, especially between different ethnics. Several studies had already observed cost-effective disparities in screening tests due to race/ethnicity differences. Guidelines from medical and professional associations such as ACR, NCCN, ASCO, CAP, and European Medicines Agency all try to specify racial or ethnic groups when providing recommendations on genotyping/phenotyping prior to using certain medications. Further efforts are needed to make more genetic tests economically worthwhile and thus to guide the incorporation of pharmacogenomics into clinical practice.

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Chapter 14
Application of Pharmacogenomics in Drug Discovery and Development

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Abstract Pharmacogenomics research aims to investigate the causes at genome level for the individual variability of drug efficacy or safety. Application of pharmacogenomics is to identify the genetic polymorphisms affecting diseases to determine the likelihood of the disease and it is influencing the drug therapy in the past decades. It is commonly applied to achieve personalized care by determining the genotype of patients in the clinical drug therapy. With the rapid progress in pharmacogenomics technologies and methods, the application has been broadened to the drug efficacy and safety studies in drug discovery and development. Pharmacogenomics can contribute to the two major determinants of the success of drug discovery and development, namely, safety and efficacy, which are more predictable by the identification of the susceptibility polymorphism of possible target and thus increase the success rate of drug development by stratifying subjects and adjusting dosage regimen in clinical trials. As there are more and more drug labels with pharmacogenomics information, global regulatory agencies have laid down the guidelines on the application of pharmacogenomics in drug development and clinical therapy. The guidance further facilitates the application of pharmacogenomics in the drug discovery and development.

Keywords Pharmacogenomics · Drug discovery · Drug development · FDA · PMDA

14.1 Introduction

Pharmacogenomics (PGx) has been established with the development of omics biotechnologies and gene sequencing and is a sub-discipline within pharmacology. Due to the genetic variations, the efficacy of the same drug may be different for the

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_14

patients with the same disease. Aiming to improve the therapeutic effect of drugs and reduce the adverse drug reactions, it is recommended to apply PGx to obtain the optimal choice of drugs and dosage in clinical practice for individualized treatment.

For the pharmaceutical industry, PGx helps determine drug targets to achieve optimal drug efficacy and good safety for the stratified subject population. The research steps of PGx are as follows: (1) According to the reported gene polymorphism and mutation in the public database, the target genetic mutants are screened by determining the genome or gene of the observed subjects; (2) To study the effect of the genetic variants on drug absorption, metabolism, efficacy, and adverse reactions; (3) To develop a gene diagnosis technology or a mutation gene diagnosis kit which can be used for judging the curative effect and adverse reaction of drugs; (4) Before patients use drugs, relevant genetic testing should be carried out to help the choice of appropriate drugs, the formulation of appropriate dosage, and course of treatment. PGx mainly focuses on the polymorphism of drug target such as metabolizing enzymes, transporters, and pharmacological receptor. By defining the sequence and expression changes of these three types of genes, we can judge the effectiveness, distribution, excretion, and toxic side effects of drugs.

Most of the candidate compounds that act on the target during the pharmacological stage cannot be successfully marketed. This is mainly due to the efficacy failure or adverse events occurring in clinical trials. In addition to individualized treatment, PGx can also be used for drug development by assessing the determinants of drug efficacy and adverse drug reactions through genome-wide survey [1]. In this chapter, we provide an overview of the application of PGx in drug discovery and development in the following sections.

14.2 Impact of Pharmacogenomics on Drug Discovery and Development

It is time-consuming from the discovery of lead compounds to the successful launch of a new drug. In 1960s, it required an average of 7.9 years for a new drug development and the average time was increased to 9–12 years in 1990s due to the complexity of the drug development process [2], which included the discovery of candidate compounds, preclinical and clinical research, and ultimate stage of marketing. In the drug discovery process, only five drug candidates may be selected from the original 5000 compounds synthesized by medicinal chemists to enter clinical phase, and only one of the five clinical drug candidates may be approved for marketing. In addition, the cost in the development of new drugs is very high. In 2000, it took about $802 million (resource cost) to develop a new drug. After capitalization, it commonly took about $1.6 million for preclinical studies, 15.2 million for clinical phase I, 16.7 million for clinical phase II, and $27.1 million for clinical phase III [3]. The most common causes of clinical trial failure are the lack of drug efficacy and the occurrence of severe adverse effects. They mainly occurs in

clinical phase II and phase III, so the cost of the financial resources will be very large [4]. As we know, the sample size of the clinical phase III is much larger than the previous two phases and so as to the larger utilization portion of resources. Failure in clinical phase III is unacceptable for pharmaceutical companies in terms of the cost of money and time. Research and development expenditure can be reduced by improving the trials of clinical phase and increasing the success rate [5].

Since the safety and efficacy of drugs are influenced by the pharmacokinetics/pharmacodynamics (PK/PD) related genes, which can be determined by PGx studies and thus the safety and efficacy can be predicted by PGx. In general, PK/PD related gene variants alter the efficacy and adverse drug effects by affecting the activity of enzymes, transporters, or drug targets. There is no doubt that the advances of PGx, including genetic sequencing technology, have expanded the application scope of PGx in various phases of drug discovery and development.

14.2.1 Impact of Pharmacogenomics on Drug Discovery

Understanding the molecular mechanisms of the complex diseases, such as obesity, asthma, and hypertension, is one of the key factors in drug development. In terms of the investigation of genetic susceptibility and phenotypic variability, PGx can affect various stages of drug development [6]. With the advances of GWAS (Genome-wide association studies) and PGx technologies, new targets can be identified by studying the genetic associations with disease and disease subtypes [7, 8]. One known example of how PGx provided major support for target identification was the development of inhibitors for the Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) [9]. The incidence of coronary heart disease in African Americans with non-functional PCSK9 variants was found to be decreased by approximately 88%. This was related to the low content of LDL cholesterol in the PCSK9 variant [10]. Thus, the PCSK9 has been identified as a novel target and translated into drug therapy, for the treatment of atherosclerotic cardiovascular disease [11].

As mentioned before, after the lead compound is identified in the process of drug discovery, it is necessary to choose the optimal clinical candidates among various compounds designed by the chemists. To screen these compounds for efficacy and toxicity, different animal models should be applied in preclinical experiments and a large amount of time and animals are required. The results of in vitro and in vivo *extrapolation* are dependent on the PK/PD similarity between the experimental animals and human.

Expression profiling at the level of nucleic acid (genomics) or protein (proteomics) can provide advantages and information for these experiments. The efficacy and profile of changes induced in the system are analyzed. Efficacy can be determined based on the desired induced changes. These tools are increasingly used to provide new understanding of interactions between the target and biomolecules and to determine the members of gene related with PK/PD of drug among gene families. For example, toxic genomics, served as a useful auxiliary tool of toxicology testing,

is increasingly applied in drug toxicity screening. Experimental data of toxic compounds are applied to create database that may reveal patterns for the prediction of the toxicity of compounds, which will help discard compounds that may experience complications in the screen process of drug discovery [12].

14.2.2 Impact of Pharmacogenomics on Drug Development

PGx can be used in retrospective or prospective studies. GWAS have assayed more than hundreds of thousands of single nucleotide polymorphisms from more than thousand individuals, and is an important PG_X tool. These studies help to discover the association of common genetic variations with more than 80 diseases [13]. In clinical development, GWAS can be used to identify candidate genes related to the PK/PD properties of compounds via retrospectively analyzing genotypic data from clinical trials. Prospectively, PGx can predict the responses to drugs by identifying the efficacy or pharmacokinetics related gene defined subgroups of patients. With the genotypic data available in the clinical phase II, it will greatly simplify the design and shorten the duration of phase III and increase the success rate [14]. Moreover, the different response to a compound of gene defined subgroup identified during early clinical phase can be applied to the development of other compounds in the same treatment area [15]. PGx provides further insights into drug efficacy and toxicity and facilitates to modulate the dosage of drug for subgroups in clinical trials. The rapid development and extensive utilization of PGx technology provide predictive tools in the clinical trials to achieve the optimal drug efficacy and reduce the adverse drug reactions (ADRs). ADRs are a major problem in clinical trials and may cause termination in clinical phase II and phase III [16]. Genomics can predict an individual's susceptibility to ADRs by determining factors that influence pharmacokinetics (polymorphic enzyme and transporter) and pharmacodynamics factors (polymorphic receptor). For instance, it is uncertain whether to make a "go" decision for drug development if only a small number of patients have a renal function change in early clinical trials. In fact there are few drugs that affect renal function in early clinical trials, whereas many drugs fail in larger clinical trials and therapy due to severe changes in renal function (especially renal failure) in some patients. In the case that serious ADRs was observed in clinical phase IV after a large number of patients had been exposed to the compound, not only the product would be withdrawn but also a large cost of time and resources would be wasted. In order to reduce the incidence rate of severe ADRs and make timely decision during the drug development, it is crucial to predict subjects who possess high risks of severer ADRs at early clinical trials using genomics. For example, whole genome SNPs profiling has enabled the determination of genetic susceptibility of ADR factors using an unbiased approach in appropriate subjects [17]. With the collection of genomic DNA from clinical trial subjects during drug development, gene polymorphisms are increasingly used for drug development by evaluating the influence of genetic polymorphisms on drug responses. In clinical development, multiple

genetic information can be obtained. Meanwhile, epidemiological racial/ethnic population control is equally effective as matched controls in phase III trials, indicating that well-defined epidemiological controls can be applied in statistical association analysis, which can overcome limited number of subject sample in early clinical trials [18].

14.3 Guidance of Pharmacogenomics in Drug Discovery and Development

In this section we will focus on the perspective of the regulatory authorities on the usage of PGx in drug discovery and drug development. Draft guidelines about the role of PGx in early clinical phase published by the US Food and Drug Administration (FDA) and guidelines about the implementation of PGx in PK experiments from the Pharmaceuticals and Medical Devices Agency (PMDA) of Japan and European Medicines Agency (EMA) are desired to make recommendations about the application of PGx from the drug discovery to clinical therapy.

14.3.1 The EMA Guideline in Drug Discovery and Development

The guideline of EMA gives suggestions to the application of pharmacogenetics in the PK trials of new drugs throughout the clinical development phases. It considers the issues about the experimental design, subject recruitment criteria, and drug dosage in the PGx related PK trials that determine the factors affect the disposition of drugs in vivo such as polymorphic cytochrome P450 and transporters, answers the question of when to evaluate the clinical influence of genetic variations on PK, and provides regulatory recommendations for the subsequent clinical trials. The main principles of the EMA guidelines on PGx related PK experiments are summarized: in which situation the consideration of the impact of PGx on PK parameters should be involved in the process of drug development; DNA sampling in PK studies; assessing the clinical consequences of genetic variation; and providing recommends based on these consequences.

The EMA guidelines indicate the necessary and recommended procedures throughout the process of drug development for PGx. When the degree of exposure changes between individuals may negatively affect the efficacy or safety of the subgroups, it is often necessary to study the impact of PGx on the PK profiles of the compound or its active metabolites and the impact on PD characteristics. If there are significant PK parameter changes among subjects and PGx cannot identify genetic factors impact the PK characteristics, effective dose can be adjusted in accordance with the genomic phenotype of the subjects in the following development phase. If

the PK study clearly indicates that the pharmacogenetics does not influence the responses to the drug, then no PGx investigation is required in the progress of clinical development. The ultimate aim of the studies in drug development is to obtain effective and safe therapy in population containing not only primary populations but also subgroups which may need dose adjustment. In the following subsections, the EMA guidelines are summarized on how to apply PGx at various phases of drug development.

14.3.1.1 The EMA Guideline in Preclinical Phase

In the preclinical studies, the in vitro metabolic pathway, enzymes, and generated metabolites should be identified. If more than half percent of the drugs is metabolized by one catalytic enzyme which is polymorphic, the EMA guidelines would believe this eliminate pathway was significant. Metabolic enzyme polymorphisms may result in reduced drug clearance, increased drug exposure in vivo, and ultimately resulting to drug efficacy and/or safety changes. The purpose of investigating PGx in this phase is to avoid unsafe exposure of drugs in subgroup with poor metabolite enzyme.

14.3.1.2 The EMA Guideline in Clinical Phase I

In order to avoid the severe adverse reactions caused by genetic variants, it is recommended to identify the types of the related gene in the population of first-in-human study when the results of preclinical trials indicate that more than half percent of the drug is eliminated by one polymorphism enzyme. If the exposure of drug or its active metabolites is predicted to increase significantly in subjects with genetic variations, the dose should be adjusted as lower for the consideration of safety in the first-in-human study. In phase I, the proportion of the clearance by the important polymorphic enzymes to the drug elimination should be investigated. The EMA recommended to study the correlation of PK parameters of drugs in the PK studies to the genotypes of the metabolic enzymes in subgroups in this phase. With sufficient literature or data available, the effects of genotypes and polymorphism can also be reflected by the results of trials adding the inhibitors of the polymorphism enzyme. In order to collect the most data for subpopulation studies in inheritance, EMA suggested to study the genotyping of the specified genes as many as possible during the drug development process if more than one-fourth drug is metabolized by single polymorphic enzyme.

14.3.1.3 The EMA Guideline in Clinical Phase II

The final goal of Phase II studies is to obtain new insights into the PK characteristics and make decision for the dosing regimen of the latter clinical trials, such as whether

to adjust dose based on genotype of the subjects. The pharmacogenetics factors affected by the pharmacokinetics characteristics obtained from PK studies in clinical phase I trials can be applied to adjust the dosing regimen in the phase II studies. If the results of the phase II study indicate that the difference in drug concentration in plasm or in a target site between common populations and subpopulations does have clinical significance, it is recommended to study individuals with intermediate metabolite phenotypes in further clinical trials. In the case that the results of the PK studies in early clinical trials indicate that it is no need to adjust dosage for the subgroups, the information of the PK results and parameters in the subgroups is also useful and can be applied for the PK studies of the clinical phase II trials.

14.3.1.4 The EMA Guideline in Clinical Phase III

If it is observed a significant difference of the PK results between the common populations with the subgroups in clinical phase II studies, EMA recommended to determine and collect the genotyping of the PK related genes and therefore adjust the dosage based on the genomic data. If the genomic factor that effects the drug PK behavior is through the transporter polymorphisms, the exposure of drug in plasma may not change in the subgroups, whereas the distribution may vary and result in a difference of the concentration of the intracellular or organ. The efficacy and safety depend on the change of the target exposure (pharmacodynamics target or toxic target). If the change is significant, it is recommended to identify the polymorphic of the related drug transporter genes and assay the impact to the PK/PD properties.

14.3.1.5 The EMA Guideline in Clinical Phase IV

Because of the relatively fewer subjects, especially the number of the subgroups in PK studies clinical phase I- III, the genomic data and information is insufficient. Therefore, rare adverse drug reactions may occur in larger size populations such as clinical phase IV or post-marketing and may cause serious outcomes. Through retrospective analysis, genomic information of other drugs in the same therapy area collected from clinical trials or post-marketing therapy can be applied to the information of drug responses (ADR or lack of efficacy) from the limited subjects in clinical trials to avoid this situation. The larger of the data (genomic data and correspond clinical response) in clinical studies, the more effective and accurate of the analysis. EMA recommends to collect and store gene samples from all subjects participating in clinical studies (I–IV).

14.3.2 The FDA Guideline in Drug Discovery and Development

Rapid development of PGx have facilitated the translation of PGx testing from lab to bedside. In the past decades, the US Food and Drug Administration (FDA) begun to recognize the importance of genetic information to the drug efficacy and safety. The drug labels were required to add recommendations related to genomic information to guide drug dosing regimen to achieve individualized medication [19]. The FDA guidance [20], published on January 29, 2013, is related to individualized medicine, and involves the application of PGx data in the early clinical trials to explore the genetic factors that affect the PK/PD behaviors, which is significant to adjust dosing regimen of the subgroups in the later clinical trials in the consideration of safety. The FDA provides guidelines for the pharmaceutical industry to submit PGx data and use of PGx data in clinical studies.

The FDA guidelines [20] are outlined as follows. It is worth noting that the guide focuses on clinical studies aimed at determining the effects of genotypes on efficacy and safety. Like other guidelines of FDA, the guide expresses insights and recommendations for the PGx application and does not have legal effect. The FDA believes that the study of pharmacogenetics in clinical trials can obtain better insight into different responses of the subjects to the new drug. In addition, the correlation between the dose and response is affected by the PD related genotype (polymorphic transporter or receptor). Related genomics data and information in the early clinical trials can be applied to the design of the dosing regimen in the later trials. The FDA guidelines recognize it is important to identify the genomic factors such as polymorphic metabolic enzyme, polymorphic transporter or polymorphic receptor that significantly affect the PK properties of the drug in preclinical studies. The information is useful to the later clinical trials to determine the correlation between the genotype with the PK/PD behaviors of the drug in vivo and the responses. It is recommended in the EMA guidelines that if more than half of the drug is cleared by enzymes that are polymorphic, the influential extent of the genotype to the change of the PK profile need to be evaluated. Such suggestions are not compulsory by the FDA because the steep concentration-response may also lead to the same outcomes. If in vitro studies indicate that drugs are mainly metabolized by polymorphic enzymes, the FDA guidance recommends to study pharmacogenetics in both single-dose PK trials and dose climbing PK trials to obtain the effect of common PK/PD related genotypes to the responses. FDA recommends to consider PK related genotypes of different ethnic or racial groups in such trials. In some cases, specialized clinical pharmacology studies may be required for retrospective analysis of such genetic variations. First, it is necessary to collect DNA information from subjects in early clinical studies to study the related genetic factors that may cause the PK changes of subgroup, and finally assay the effect degree of the genetic cause on PK properties. If the exposure is significantly increased in subgroups of subjects due to the change of clearance caused by the genetic variations, FDA recommends a prospective determination of genotype to decide whether make dosing adjustment

or redefine recruitment criteria for the subjects in the early clinical studies. Active metabolites generated by polymorphic enzyme may also lead to exposure difference and influence the responses to drug. PK/PD related genetic variations can be collected and assayed using PGx methods such as high throughput approaches and targeted gene methodologies in clinical phase I/II trials. If significant PK/PD related genetic variations are determined in early clinical trials, FDA recommends to study the effect degree of PGx to PK/PD in the later clinical trials and adjust dosing regimen for the subgroups. For such case, relevant genetic variations and PK/PD information should be collected as many as possible for the later clinical trial design. According to such information, physicians and researchers can develop recruitment criteria, group the subjects, and adjust the dosing regimen to reduce the incidence of adverse reactions and increase the possibility of success of clinical trials. FDA recommends to study the correlation between genotype with the relationship of dose/concentration-response, which is also the focus of the PGx study in the clinical trials. It is recommended to further study the effect degree of the genetic variations to the dose/concentration-response relationship in the later clinical trials if the correlation is indeed observed in early clinical trials.

14.3.3 The PMDA Guideline in Drug Discovery and Development

The publication and modification of PMDA guidelines promotes the application of PGx in drug development [21]. The views and recommendations of the PMDA for the application of the PGx in drug development are outlined below. The PMDA guidelines recommend to examine the effects of polymorphism of metabolic enzymes on PK properties and DDI. In the PK or DDI study, genetic testing is recommended to identify the genotype and stratify subjects to choose appropriate populations for further clinical studies when the drug PK parameters have significant individual differences and/or the drug is primarily eliminated by the polymorphic enzyme. PMDA does not set a threshold for the significant effect of polymorphic metabolic enzymes on drug clearance and does not specify when to study the relationship of PGx with PK properties in clinical trials. But after the publishing of the PMDA guidelines, the experience and understanding about the application of PGx in drug discovery and development achieve rapid advance. Applying pharmacogenetics in global drug development is also significant. When PK studies found that the genetic variability of metabolic enzymes or transporters significantly affected efficacy/adverse reactions, PMDA encouraged to analyze PGx in clinical trials of global multi-hospital center to characterize the difference of inter-ethnic genetic variation and the relationship between gene phenotype and PK characteristics. When the results show a large distinct between the PK characteristics of inter-ethnic subjects, the inter-ethnic difference of genotype may partly answer the issue and guide the design of the dosing regimen for different race.

Table 14.1 Differences between the guidelines of EMA, FDA, and PMDA on PGx

Issue	EMA	FDA	PMDA
Suggested application of PGx in phases of drug development	Preclinical phase and clinical Phases of drug development (focused on PK studies)	Mainly on early clinical phases (I and II)	Clinical phases (mainly on PK or DDI studies in Phases I–IV)
In vitro threshold	If more than half of the drug is cleared by enzymes that are polymorphic in preclinical studies, the effect degree of the genotype to the change of the PK properties need to be studied	Such suggestions were not proposed by the FDA because the steep concentration-response may also result to the same outcomes. If in vitro studies indicate that drugs are mainly metabolized by polymorphic enzymes, the FDA guidance recommends to study pharmacogenetics in both single-dose PK trials and dose climbing PK trials to obtain the effect of common PK/PD related genotypes to the responses. FDA recommends to consider PK related genotypes of different ethnic or racial groups in such trials.	PMDA does not set a threshold for the significant effect of polymorphic metabolic enzymes on drug clearance.
In vivo threshold	If more than one-fourth drug is metabolized by single polymorphic enzyme, EMA suggested to study the genotyping of the specified genes during the drug development process.	No suggestions	PMDA does not specify when to study the relationship of PGx with PK properties in clinical trials.
DNA information collected	EMA recommends to collect and store gene samples from all subjects participating in clinical studies (I–IV)	Recommended	Recommended

In summary, the differences between the guidelines of EMA, FDA, and PMDA on PGx issues are shown in Table 14.1.

14.4 Application Cases of Pharmacogenomics in Drug Discovery and Development

The principles of application of the PGx in medical treatment should comply with the following guidelines: (1) it has been ascertained that the genotype affect the drug responses; (2) the genomic data should be tested and collected; (3) the individual gene data should be confidential in accordance with personal wishes. The role of the PGx in every phase of drug development [5] are listed in Table 14.2.

14.4.1 Application of Pharmacogenomics in Drug Discovery

Before the drug development, it is necessary to identify and discover the potential targets (activity or toxicity). The individual responses (drug efficacy/safety) to the drug are largely affected by the PK/PD targets such as the metabolic enzyme, transporter, and receptor (active or toxic target), the activity of which may be changed due to genetic variants. For instance, there are two kinds of genotype of β2 adrenergic receptor, one of which is active, and the other is inactive [22]. It is necessary to avoid such polymorphic targets and select other appropriate targets for drug discovery. Commonly, the difference of individual response to drugs is originated from multiple genes instead of one single gene. Therefore, the target can be identified on the basis of PGx information to select pharmaceutical compounds acting on an appropriate target for further investment in the process of drug discovery. Appropriate target can be predicted using computational models trained with

Table 14.2 Role of PGx in every phase of drug development

Stage	Application of PGx
Drug discovery	Identification and assessment of the polymorphic of the drug target
Phase I clinical trial	Subject selection—Identification of the genotype of subjects and making inclusion/exclusion criteria
	Dose regimen ascertain
Phase II clinical trial	Dose regimen adjustment based on the genotype of the patients
Phase III clinical trial	Assaying the difference of drug efficacy and adverse drug reactions based on genetic variants
Phase IV clinical trial	Interpretation of reported adverse events with PGx information
Regulatory requirements	Submission of PGx data during drug development
Patient therapeutics	Personalization of drug therapy
	PGx information in drug labeling
	Identification of the stratified population of non-responders and high risk groups of adverse events

data of genome sequences of human population. For example, it is identified that the occurrence of coronary artery disease is associated with the genetic mutations of P2Y12 receptors of platelets, leading to the recognition that P2Y12 receptors may be a suitable target for pharmaceutical compounds for the treatment of coronary artery disease [23]. There are about 8000 targets, among which 4990 can be acted by drugs and 794 are proteins, in the current available human genome sequences and 399 targets of molecule have been identified in protein families [24, 25]. Some cases for targets identification and selection are described below.

14.4.1.1 Alirocumab and Evolocumab

Atherosclerotic cardiovascular disease (ASCVD) is one of the diseases with the highest mortality rate though the rapid development of modern pharmacotherapy. The main therapy target for ASCVD is lower density lipoprotein cholesterol (LDL-C). The development of inhibitors for the PCSK9 saga might be one of the best examples for the application of PGx in the drug discovery [26]. In 2003, it was first identified the gain-of-function (GOF) mutations in PCSK9 to cause severe autosomal dominant hypercholesterolemia in a cohort of French families [10]. Later, Hobbs and Cohen found PCSK9 LOF mutations could lead to a reduction of 28–40% in LDL-C levels and much lower incidence rate of coronary artery disease than the incidence rate in the wild genotype of an African-American population of large size [27, 28]. The strong pathophysiological association between the LDL-C reduction and PCSK9 mutation prompted scientists to investigate PCSK9 as a drug target for the treatment of ASCVD by various experiment approaches such as protein inhibitors, EGF-A inhibitors, translation/RNA inhibitors, adnectin inhibitor, and monoclonal antibiotics as well [29]. It took less than 5 years for PCSK9 to reach the phase of target validation since its initial discovery and then rapidly translated to the phase of novel candidate compounds discovery and drug development. Among which, two typical examples are alirocumab and evolocumab, both PCSK9 inhibitors. Alirocumab was developed by Regeneron pharmaceuticals and Sanofi for additional LDL-C reduction for the treatment of heterozygous (HeFH) or ASCVD (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm455883.htm). It is reported that the LDL-C was impressively decreased within 66.2–73.2% for patients administered with alirocumab [30–32] and adverse reactions were comparable with placebo except for injection site reactions. On July 24, 2015, alirocumab (Praluent®) received FDA approval. Evolocumab was developed for the treatment of HeFH or indications that cannot reduce LDL-C by statins and prevention of adverse cardiovascular events (MACE) in ASCVD patients by Amgen. It was shown that the level of LDL-C was lowed 75% for patients administrated evolocumab and adverse reactions were also comparable with placebo. Injection site reactions were 3.3% for evolocumab group vs 3% for placebo group [29]. On August 27, 2015, evolocumab (Repatha®) received FDA approval [33].

14.4.1.2 Imatinib

The oncogene of encoding BCR-ABL (V-abl Abelson murine leukemia viral-breakpoint cluster region) protein can constitutively activate the activity of tyrosine kinase, which could cause chronic myeloid leukemia (CML). The induction of the oncogene is related with the chromosomal translocation of ABL gene on chromosome 9 onto the BCR gene on chromosome 22. This carcinogenic mechanism indicates that the tyrosine kinase domain of BCR-ABL can be target of CML. Imatinib was discovered to inhibit this target based on the above genomic information and was one of the first targeted anticancer drugs rather than nonspecifically acting on all the cells of rapid division. The development of imatinib and application in the therapy treatment have achieved great success. It is reported that the rate of cumulative complete cytogenetic response in patients was accounted for 83% and achieve 85% survival rate during the clinical therapy [34]. Imatinib was also found to be related with the KIT tyrosine kinase receptor and successfully apply to the treatment of gastrointestinal stromal tumors induced by mutation of KIT [35].

14.4.1.3 Trastuzumab

About one-fourth or more breast cancer patients present HER2 overexpression or amplification, which may cause adverse reactions such as cancer recurrence, aggressive tumor, relative resistance of anticancer, and poor prognosis [36, 37]. This genomic information provides the appropriate target, which is HER2, for breast cancer treatment. Trastuzumab was the first targeted drug for the treatment of breast cancer and the extracellular domain of HER2 receptor is the specifically acted site of this drug. Thus before the treatment, it is necessary to test if the gene of HER2 is overexpression or amplification to select appropriate patients for best clinical beneficial.

14.4.2 Application of Pharmacogenomics in Clinical Development

Application of PGx tools into various phases of clinical trial is helpful in drug development. In addition to the selection of appropriate targets, it is important to identify whether polymorphic factors significantly influence the PK/PD properties of compounds through in vitro/preclinical studies and make "Go" or "No Go" decisions for further trials [38]. If clinical trials are desired, the genomics information obtained in preclinical studies is useful for the design of the recruitment criteria of subjects and dosing regimen to prevent the occurrence of adverse drug actions and increase the possibility of drug development success [39]. It needs to point out that the subjects recruitment criteria can be designed based on polymorphic factors only in

case that such factors are related with the PK parameters. The PGx information should also be collected in early clinical phases and is helpful for the further drug development.

The main reasons for drug development failure are poor efficacy and adverse drug actions. Except for PGx methods, there are currently no particularly effective tools to predict these two factors. Development failures in clinical trials can result in severe economic losses. With the development of genetic genomics and the use of complex pharmacogenetics methodologies, the failure in the clinical studies can be partly avoided, and the economic losses in drug development can be saved. Application of PGx in clinical trials may partly avoid the severe adverse reaction and predict safety in different subgroups stratified based on genotypes. For example, subjects of poor metabolic capacity due to gene phenotype tend to achieve higher drug plasma concentrations and higher possibility of toxicity. Allopurinol is a commonly used xanthine oxidase inhibitor for the treatment of gout or hyperuricemia, and the improper use may lead to serious skin reactions. In patients taking allopurinol, 100% of those who developed adverse skin reactions had the genetic variant of *HLA-B∗5801*. Therefore, before treatment with allopurinol, patients should be tested for *HLA-B∗5801* genotype carriers.

14.4.2.1 Trastuzumab

Trastuzumab is an antibody that against HER2 metastatic breast cancer monoclonal. During the early clinical phase of drug development process, the drug was found to act only in women with overexpressed HER2 protein, implying such subjects should be recruited in the further clinical trials [40, 41]. Trastuzumab was approved to treat overexpressed HER2 patients who needed gene testing. If the genotype of the subjects were not identified and the entire population rather than population only overexpressed HER2 was recruited in the clinical trials, efficacy would not be found. It will lead to the termination of drug development when there are severe adverse drug reactions observed in clinical trials.

14.4.2.2 Statins

Statins are widely used lipid-lowering drugs for the treatment of hypercholesterolemia and for preventing of coronary atherosclerosis-related diseases. Statins are generally safe, but occasionally have adverse reactions, such as rhabdomyolysis, a disease in which kidney damage is caused by toxic substances produced by muscle cells, commonly known as myolysis. At 80 mg/day, approximately 0.9% of patients develop myopathy. A mutation in the SLCO1B1 gene causes the protein it encodes to increase its ability to bind statins in the liver, leaving too much of the drug in the body [42]. The study showed that people carrying two SLCO1B1 gene risk markers had a 15% probability of adverse reactions when using statins, while non-carriers had a 0.3% probability of adverse reactions.

14.4.2.3 Atomoxetine

Attention deficit hyperactivity disorder (ADHD) is a neuropsychiatric disease that commonly happens in childhood with an estimated prevalence of 8–10% [43, 44]. Atomoxetine (Strattera®), a selective norepinephrine reuptake inhibitor, was the first novel nonstimulant drug approved by FDA for the treatment of ADHD in 2002. Atomoxetine is primarily cleared by the liver and is the substrate of liver cytochrome P450 (CYP) 2D6 (CYP2D6). It is reported that the plasma clearance of atomoxetine was 0.35 L/h/kg in EMs, which was more than tenfold greater than that of 0.03 L/h/kg in PMs, the area under the curve (AUC) of concentration versus time in PMs was about tenfold greater than that in EMs and the maximum plasma concentration (Cmax) in PMs was fivefold greater than that in EMs [45]. Taken into consideration that the polymorphic of CYP2D6 gene greatly affect the individual pharmacokinetics of atomoxetine, the PGx information in the clinical trials of atomoxetine was collected and a retrospective analysis was processed. The results showed that the incidence rate of adverse reaction in PM subjects was twice than that in EM subjects (Table 14.3). The label was updated and recommended to adjust dose regimen in patients of CYP2D6 PMs in 2011 [46].

14.4.3 Application of Pharmacogenomics in Post Drug Approval Phase

Adverse reactions were mainly due to polymorphisms in metabolic enzymes. The most common application of pharmacogenetics in clinical therapy is the detection of individual genotype of polymorphic drug-metabolizing enzymes/transporters/receptors, especially enzymes, to adjust the dose. As applying pharmacogenetics tools in drug development is relatively new, many labels of drug have updated with genetic information after drug approval.

Table 14.3 Incidence rate of adverse effects of atomoxetine in PM subjects and EM subjects

	Incidence rate	
Adverse effects	PMs (%)	EMs (%)
Decreased appetite	23	16
Insomnia	13	7
Sedation	4	2
Depression	6	2
Tremor	4	1
Early morning awakening	3	1
Pruritus	2	1
Mydriasis	2	1

14.4.4 Carbamazepine and Abacavir

Carbamazepine is a preferred drug for the treatment of epilepsy, but also for the treatment of trigeminal neuralgia, bipolar disorder, and arrhythmia. Carbamazepine can cause severe or even fatal skin reactions. In the Asian population, this adverse reaction occurs in 1–6 people per 1000 people, because the patient has the human leukocyte antigen allele HLA-B $*$ 1502. The FDA recommends that patients, especially Asian patients, be tested for HLA-B $*$ 1502 before their doctors prescribe carbamazepine (including similar products). Carbamazepine should not be used in positive carriers.

Abacavir is a nucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus (HIV) infection. Hypersensitivity reaction was reported in few patients for repeated usage of abacavir and leaded to black box warning. A program of post-marketing pharmacovigilance was built for this situation. It is found to be related with HLA-B*5701, a pharmacogenetics marker commonly exiting among different racial population groups [47], through prospective, double-blind, gene-guided, randomized and observational clinical trials. The patients are recommended to test HLA-B$*$5701 before the treatment of abacavir to reduce the reaction of hypersensitivity by the FDA during 2008. The drug labels were approved to update by the European Union in 2008, with the suggestion of screening the genotype of HLA-B$*$5701 prior to abacavir therapy for HIV treatment.

14.4.4.1 Aminoglycoside Antibiotics

Aminoglycoside antibiotics are commonly used in severe infections caused by Gram-negative bacteria, such as meningitis, respiratory tract, skin, trauma, and so on. Such antibiotics include streptomycin, gentamicin, kanamycin, neomycin, etc. Improper use can lead to serious drug-induced deafness in patients. A large number of studies have confirmed that mitochondrial DNA 12S rRNA gene A1555G or C1494T gene mutations are the main culprits of sensorineural hearing loss caused by aminoglycoside antibiotics [48].

14.4.4.2 Monoclonal Antibodies

Cetuximab and panitumumab are monoclonal antibodies for colon cancer. It is indicated that the drug efficacy in the patients of KRAS mutations were lower from retrospective analysis [49]. The panitumumab was approved for the colon cancer treatment in patients with wild KRAS genotype during 2007. The drug labels have approved to update the pharmacogenetics information by the FDA in 2009.

Application of pharmacogenetics tools in drug development can improve the drug efficacy in the appropriate patients, thus reduce the ADRs of drugs and finally raise

the success possibilities of drug development. Pharmacogenetics increases the launch rate for the uncertainty novel drugs and the usage in the therapy. More and more drugs were approved to include PGx information in drug labels. FDA [50] listed 286 approved drugs with PGx information in drug labeling (accessed on 3 September 2019).

14.5 Conclusion and Perspective

Inter-individual differences of drug efficacy may result from the changed activity of the polymorphic targets such as enzymes, transporters, and receptors. Pharmacogenetics techniques and tools are applied in every phases of drug discovery and development with the rapid advances of PGx to guarantee safe dosing regime and increase success possibility, thus reducing the losses of financial resources. DNA samples are encouraged to be collected for further studies and medical treatment. Currently, regulatory authorities also recommend to add PGx information in drug labels to optimize the drug usage and achieve individualized medicine. More and more drugs were approved to include PGx information in drug labels. FDA has listed 286 approved drugs with PGx information in drug labeling (accessed on 3 September 2019) [50].

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Chapter 15
Barriers and Solutions in Clinical Implementation of Pharmacogenomics for Personalized Medicine

Zhaoqian Liu, Xi Li, and Boting Zhou

Abstract Nowadays, it is the trend for medical development to develop personalized medicine, and pharmacogenomics is one of the main driving forces. In recent years, with the rapid development of pharmacogenomics, increasing biomarkers related to drug efficacy and toxicity have been discovered, which makes individualized treatment in clinic become possible. Using these biomarkers to guide clinical medication is expected to improve the efficacy and safety of some drugs, and reduce side effects and medical costs. Although a lot of drug labels have been identified based on pharmacogenomics research, only a few drugs used in clinic have considered its pharmacogenomics information. There are still many barriers in the widespread promotion and application of pharmacogenomics research results in clinical practice. Recently, the major barriers included information technology, scientific research, education, ethnic differences as well as policies and regulations. In this chapter, we will detail the major barriers associated with the clinical application of pharmacogenomics and their possible solutions.

Keywords Pharmacogenomics · Barriers · Clinical applications

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W. Cai et al. (eds.), *Pharmacogenomics in Precision Medicine*,
https://doi.org/10.1007/978-981-15-3895-7_15

15.1 Introduction

In recent years, personalized medicine has become a major trend, and accurate individualized treatment is expected to greatly improve the treatment success rate of the disease, reduce the incidence of side effects, and better protect the health and life safety of patients. The ultimate goal of personalized medicine is to give the right patient the right treatments at the right time according to the patient's genetic and environmental background [1]. Pharmacogenomics is an interdisciplinary which combines genomics and pharmacology to investigate the role of the genome in drug response, and it is one of the most important driving force of personalized medicine.

Pharmacogenomics is developed on the basis of human genome project and inherited the characteristics of pharmacogenetics which is first proposed by Vogel in 1959 [2]. At present, pharmacogenomics plays an important role in the discovery of biomarkers and the development of new drugs. Based on pharmacogenomics study, researchers can identify genetic factors related to drug response or adverse reactions, and develop an individual therapy method to improve the clinical effect of drug treatment [3–6]. Through the use of pharmacogenomics, the adverse drug reaction (ADR) can be reduced, the number of drug clinical trial failures can be reduced, the time required for drug approval and the duration of drug administration can be shortened, and the number of drugs taken and the impact of the illness on the body can be reduced [6]. Therefore, the overall costs of medical treatment and public health could be greatly reduced [6]. One of the most famous example of clinical implementation of pharmacogenomics is reducing the ADR of the patients who received carbamazepine by screening of *HLA-B ∗1502* [7, 8]. The *HLA-B ∗1502* allele was associated with SJS and TEN after carbamazepine treatment, and changed the treatment regimens of the carriers of *HLA-B ∗1502* significantly reduced the ADR of carbamazepine [7, 8].

With the development of pharmacogenomics, more and more governments begin to pay attention to the influence of genetic factors on drug response. As early as in 2005, the US Food and Drug Administration (FDA) issued "Pharmacogenomic data submissions," a guidance for industry [9]. This guidance guides the drug research and development manufacturers how and when to submit genomic data to the FDA. Moreover, in the last few years, FDA and the European Medicines Agency (EMA) have added a number of clinically validated pharmacogenomics biomarkers in drug labeling [10]. To date, a total of 82 biomarkers are added in the "Table of Pharmacogenomic Biomarkers in Drug Labeling" according to 270 FDA approved drugs (https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling). Although about 15% of FDA and EMA-approved drugs have pharmacogenomics information on their drug label, only a few drugs used in clinic had considered its pharmacogenomics information [11]. In actual clinical applications, the progress of pharmacogenomics implementation is very slow [12]. Identifying the barriers that limit the clinical application of pharmacogenomics and suggesting solutions will strongly advance the process of pharmacogenomics, which will help lay a solid foundation for individualized treatment.

15.2 How to Apply Pharmacogenomics to Clinical Practice

The origins of pharmacogenomics can date back to 510 BC, when Pythagoras first found that some people had fatal hemolytic anemia after eating broad beans. This reason was later proved by the genetic defect of glucose-6-phosphate dehydrogenase (G6PD). This deficiency resulted to a decrease in the activity of G6PD, which is unable to produce sufficient reducing NADPH to protect red blood cells against oxidative stress, and ultimately lead to an acute hemolysis reaction when those people exposure to some oxidizing substance or drugs. There are 21 different clinical annotations for G6PD variations that currently reported in PharmGKB [9], which is a pharmacogenomics knowledge recourse offering potentially clinically actionable gene-drug association and gene-phenotype relationships. Besides, CPIC is another pharmacogenomics database for medical workers to find the interpretation of the clinical implementation of the pharmacogenomics tests for making actionable prescribing decisions [13, 14]. They are two successful cases of pharmacogenomics transformation in the clinic. Today's pharmacogenomics development built on the human genome-wide program and genome-wide genetic variation detection technology. Researchers use those technology to conduct their studies on finding the correlation between the genotypes and specific drug effect phenotype (such as efficacy and adverse effects) [15]. The current research strategy is to figure out the reasons that some drugs only benefiting some patients, while the remainder are ineffective, some drugs can even cause severe side effect in a small proportion of patients while not affect others. Researchers compare and identify genetic variant sites and/or genotypes for the two groups of patients by using GWAS to find the correlate with drug effects [16]. Those result can help to provide precision medicine by only prescribe the drug to the patients who will respond to the drug, and suggest patients who may not respond to this drug or may suffer some severe side effects to change other drugs [17]. To apply pharmacogenomics into better clinical practice, European and North American countries have already gain wider experience which can give us a lot of insight to learn. In short, there are many steps to achieve successful clinical practice, including adopting a pre-emptive strategy, promoting integration equipment, establishing a monitoring committee, conducting strict quality control and keeping update the information, continuously perfecting the clinical decision support system, last but not least, establishing the continuing education programs for doctors [18].

15.3 Barriers in Clinical Implementation of Pharmacogenomics

With the concept of precision medicine being gradually accepted by public, the application of pharmacogenomics research results in clinical practice is increasing. Inevitably, the application process faces obstacles from different levels, which will

affect the development of personalized medicine more or less. There are five kinds of barriers that we are facing generally: information technology, scientific research, education, ethnic differences, as well as policies and regulations. Except to the five key barriers, factors that may affect the clinical application of pharmacogenomics are also based on national circumstances, such as different regulatory frameworks for pharmacogenomics testing and differences in social perceptions across countries [11].

15.3.1 Information Technology

The clinical application of pharmacogenomics relies on information technology, especially on the integration of various clinical information systems and big data technologies [19]. There is still a huge gap between the current genomic results and the clinical practice of personalized precision medicine. One of the most important reasons is lack of strong clinical research evidence, which is caused by difficulties in clinical data collection, small sample size, the low reliability and verifiability of the research results, and so on. To solve these problems, the construction of a large and extensive information infrastructure to collect patient samples and clinical data is required to further develop and integrate clinical networks of open storage resources for comparative study of genomic and precision medicine outcomes. However, with China as an example, there is a big gap between the development requirements of bioinformatics and the quality of medical information management students at present, such as medical professional knowledge, computer skills, and data processing ability. Moreover, the overall planning for the construction of health information system is lacking, and the electronic case system of hospitals is incompatible. Therefore, all regional health information systems are still isolated to each other. Data cannot be shared and valuable medical data cannot be formed.

15.3.2 Scientific Research

Difficulties in scientific research are the most common barrier of the clinical application of pharmacogenomics. There are three aspects to this problem, including the credibility of the test results, the reliability of research results, and the complexity of interpretation of the research results. Recently, scientists are constantly trying to develop new genotyping tools, and hope that the new tools can be easily applied to the treatment of clinical drugs [11]. However, evidence studies that demonstrate the feasibility and clinical utility of these genotyping techniques and tools are still lacking [3, 18, 20]. In addition, the length of the cycle for certain treatment options can be problematic because some medical institutions may not be able to complete the test in a relatively short time [21, 22]. Moreover, recent clinical pharmacogenomics research lacks prospective, large sample, multi-center randomized

controlled trials (RCT), and often leads to inconsistent results, making it difficult for pharmacogenomics to be effectively transformed into clinical [22]. As a result, the acceptance of individualized treatment based on pharmacogenomics by clinicians and patients is still low [23]. At the same time, with the development of genotyping chips and pharmacogenomics detection kits, should single gene be analyzed in a clinical application or should a group of genes be detected? And how to interpret the results in opposite directions when considering multiple biomarker with different effects? Finally, clinical patients rarely use a single drug, and there are interactions between multiple drugs. But most of biomarkers are used for predicting the response of a single drug. It is difficult to evaluate the effects of various influencing factors in a single way, which makes scientific research more difficult.

15.3.3 Education

Lack of education and training is one of the biggest factors affecting the clinical implementation of pharmacogenomics. The expertise of pharmacogenomics is needed for clinicians, patients, and other relevant personnel in the implementation of personalized precision medicine. However, there is no pharmacogenomics course in the clinical medicine education at present in most of university. Due to widespread lack of the expertise of pharmacogenomics and precision medicine, physicians and patients do not recognize the importance of pharmacogenomics information in developing optimal treatment options, even if after participating in the course of clinical application of pharmacogenomics [24–27]. Moreover, doctors lack systematic and comprehensive knowledge of pharmacogenomics to guide the professional use of drugs, which also leads to the clinical application of pharmacogenomics not able to the extent and effect that they are expected [27].

15.3.4 Ethnic Differences

The clinical application of pharmacogenomics also faces obstacles caused by ethnic differences. For a long time, clinical medicine has always applied the same dose for the same disease. Clinicians have administered doses based on local ethnic populations in the countries where the medicine was developed. In most of races, drug dosage is basically used according to Caucasian dosage standards. Due to ethnic differences, a considerable proportion of patients may have poor drug efficacy or side effect. The earliest experimental evidence on racial differences in drug response confirmed that normal Chinese men are at least twice as sensitive to propranolol β-blocking effect, that is, white people should have more than twice the plasma propranolol concentration than Chinese, to produce the same beta-blocker effect [28]. Most of racial differences can be explained by genetic factors. Take warfarin, for example, the average dosage for Chinese and Caucasian are about

3 mg/day and 5 mg/day, respectively. Previous study confirmed that the *VKORC1 -1639 G>A* polymorphism are significantly associated with warfarin dose, and the carriers of G allele have higher dose than the carriers of A allele [29]. In Chinese, the frequency of G allele is about 10%, while in Caucasians, the frequency of G allele is more than 55% (according to Ensemble database). Therefore, when foreign studies found new genetic polymorphisms related to drug metabolism, effects, and safety, they could not be directly applied to domestic patients, which increased the workload and difficulty of clinical application of pharmacogenomics.

15.3.5 Policies and Regulations

Imperfect policies and regulations are another important barrier, and different countries face different problems. Taking China as an example, gene-oriented individualized treatment faces a lot of constraints. First of all, from the perspective of social ethics, if the patient is not fully informed of the significance of detection, it may aggravate the contradiction between doctors and patients to some extent. Because genetic testing has not yet become a clinically common test, if the functional gene of the patient is not missing, it can still be administered according to the conventional dose, and the patient and his family are likely to have excessive medical suspicion about the doctor [30]. On the other hand, gene sequencing results involve personal privacy, and the disclosure of personal genetic data may threaten the privacy of the entire family and even cause social discrimination [31]. Second, the field of drug gene detection in China is at the development stage, and relevant guidelines and regulations are still not perfect. For example, the scope of application and the type of patients for genetic testing are not specifically described in the newly published "Catalogue of Clinical Laboratory Items for Medical Institutions" that is formulated by National Health Commission of China [32]. The "Technical Guidelines for Gene Detection of Drug Metabolizing Enzymes and Drug Targets" does not clearly define the recommended level for individualized drug gene testing projects, and cannot meet the actual clinical needs [32]. Moreover, the quality of commercial testing institutions is mixed, but the regulatory policy is not perfect. There are very few commercial genetic testing laboratories with laboratory certifications issued by the Clinical Testing Center of National Health Commission of China.

Medicare reimbursement is considered to be another important barrier that needs to be overcome in clinical applications of pharmacogenomics. Recently, due to lack of evidence of effectiveness and incentives, the limit of research data on the cost-effectiveness of genetic testing, and the low clinical feasibility of many pharmacogenomics tests, the Medicare reimbursement only include a few of pharmacogenomics test items at present [21]. Therefore, patients need to do some drug genomic tests at their own expense [12, 33]. Due to the consideration of saving medical expenses, patients are only interested in pharmacogenomics testing at reasonable prices. In fact, most of patients will not choose the pharmacogenomics test unless it is necessary. Moreover, after the test results are added to the electronic

case, the doctor can obtain the corresponding information. However, if the test result is not reliable, the doctor may mis-select the non-optimal dose of the drug, resulting in adverse effects such as poor treatment or increased medical costs, and the insurance company may have to pay more. As a result, only the pharmacogenomics tests that have been confirmed cost-effectiveness will be included in the insurance. Unfortunately, due to the difficulty of the study, only a few pharmacogenomics tests conduct cost-effectiveness study.

15.4 Feasible Solution

In order to better apply pharmacogenomics to clinical practice and provide patients with more accurate and effective treatment options, this section will propose possible solutions of the problems raised in the previous section.

15.4.1 Information Technology

The clinical application of pharmacogenomics relies on information technology, especially on the integration of various clinical information systems and big data technologies. The complete development of the pharmacogenomics clinical decision support system (PGx-CDS system) are the pivotal issue, which will enable the clinical application of pharmacogenomics to proceed smoothly [34]. CDS system is a clinical decision support system, which can provide timely decision-making assistance for doctors, patients, and other stakeholders by combining the latest scientific knowledge and the patient's condition [35]. It is a vital tool to overcome many obstacles and challenges encountered in the clinical implementation of precision medicine and to promote the realization of precision medicine [36]. Among them, the CDS system for the purpose of helping and promoting the use of pharmacogenomics knowledge by clinical staff is called the PGx-CDS system. Due to the characteristics of pharmacogenomics knowledge, the PGx-CDS system is indispensable for clinical deployment and knowledge transformation of pharmacogenomics [37, 38].

In addition, to overcome the low reliability of pharmacogenomics research results caused by the difficulty in collecting clinical samples, we also need to strengthen the collection and management of patients' biology and register samples. A networked and standardized pharmacogenomics BioBank system should be developed to match the collection of patient samples with the contents of patient cases. A stand pharmacogenomics BioBank system are needed for high quality pharmacogenomics research and testing.

15.4.2 Scientific Research

As pointed out in the previous section, the biggest problem in pharmacogenomics scientific research is the lack of supporting evidence for the credibility of the test results [39]. This problem is related to several aspects: first is the problem of genetic detection technology. We need to develop effective methods and low-cost genotyping technology. Reduced costs and increased speed make it possible to target the genetic makeup of ordinary individuals and help to obtain more clinical data. Second, due to the lack of large sample multi-center RCTs, existing evidence-based medical evidence cannot be simply understood as pharmacogenomics-oriented treatment will improve clinical outcomes. Therefore, we need to do a lot of large sample clinical research and evaluate the evidence base to form a reliable medication guide. Finally, for the complexity of pharmacogenomics caused by the interaction of various targets, we can carry out real-world research to obtain test results that are more similar to the clinical reality, and to guide clinical medication.

15.4.3 Education

The lack of education and training for medical practitioners has hindered the clinical application of pharmacogenomics. Most medical professionals agree with the personalized administration based on pharmacogenomics guidance, but they are not confident in combining pharmacogenomics knowledge to answer patient counseling and provide medication guidance, largely because pharmacogenomics knowledge is not included in the original professional curriculum [40]. Regarding this problem, strengthening and improving genetics education in medical schools is required. Qualified schools can carry out courses on pharmacogenomics among undergraduates and graduate students, and educate them on basic theories of personalized precision medicine. We also need to strengthen the training of clinicians on pharmacogenomics and personalized medicine in order to integrate them with existing clinical information and guide clinical practice [41]. These measures will enable medical students and doctors to have a certain degree of theoretical knowledge, and to have a certain degree of acceptance and recognition when they encounter pharmacogenomics knowledge related to personalized medicine in clinical work [41].

In addition, we must also strengthen the training of clinical pharmacists. Clinical pharmacists are the executors who use pharmacogenomics theory to guide clinical drug use, and are the main force to promote rational drug use and reduce adverse reactions [42]. In the 1940s, the clinical pharmacy specialty was established in the USA, which changed the cultivation of pharmaceutical talents from “drugs” to “people” [43]. Nowadays, nearly one hundred colleges and universities are qualified to train clinical pharmacy professionals [44]. In China, many famous universities

offer clinical pharmacy course for undergraduate students, such as Sichuan University, Central South University, China Pharmaceutical University, and so on.

Moreover, in addition to theoretical knowledge, other knowledge such as, precision medicine related policies and regulations, genotyping technology, and quality standards are also need to be trained. This process promotes individual medical detection technology specification management, and ensure the quality of clinical laboratory services and medical security.

15.4.4 Ethnic Differences

Because different alleles occur at different frequencies in different races, racial differences in pharmacogenomics are also a major reason hindering personalized medicine. In addition, because of lack of basic pharmacogenomics data in Chinese population, racial differences make it impossible for European and American population data precise guidance for Chinese patients to take medicines. The premise of large-scale development of pharmacogenomics in China is the need to build a basic database with characteristics of the Chinese population. Therefore, the clinical implementation of pharmacogenomics must be based on the clinical data and follow-up information of Chinese people, and the actual medication guidance suitable for the Chinese population should be developed through pharmacogenomics researches.

15.4.5 Policies and Regulations

For ethical constraints, the standard informed consent is needed. Effective informed consent and independent ethical review are the two pillars that safeguard the rights of research participants [45]. In response to the difficulties encountered in informed consent, we can standardize effective informed consent requirements and set up a special informed consent advisory department to offer patients, subjects, and sample donors the comprehensive, accurate information they need to make decisions. In addition, gene sequencing results involve personal privacy, and we must balance the protection of personal privacy with the full use and wide sharing of databases [42]. First, it requires a safe way to store samples, data, and other relevant information. Second, we must control the accessibility of these stored samples and data. In addition, the field of drug gene detection in China is at a development stage, and the relevant guidelines and regulations are not perfect enough to meet the actual clinical needs. For government decision-making departments, it is necessary to speed up the introduction of relevant policies to protect the privacy of patients and encourage the innovative development of genetic testing technology [46, 47].

Pharmacogenomics testing and treatment are not included in reimbursement by a great number of insurance companies, and public health insurance in various

Table 15.1 Comparison of medical insurance in different regions

Region	Medical insurance policy
USA	On March 16, 2018, the US CMS (Medical Insurance and Medicaid Services Center) announced that nationwide advanced cancer patients can be reimbursed for diagnostic laboratory testing using next-generation sequencing technology (NGS)
Europe	Germany, the United Kingdom, Ireland, Spain, and Switzerland have included the Oncotype DX® Breast Cancer Recurrence Risk Assessment Genetic Testing Product (Breast Cancer 21 Genetic Testing) in the reimbursement system
China	Since June 15, 2019, Beijing has included tumor gene detection in medical insurance for the first time, and the reimbursement rate is as high as 90% Patients can get genetic testing to meet clinical drug decisions in the hospital and enjoy medical insurance reimbursement Before using targeted drug, compliance genetic testing is required before reimbursement of drug costs

countries. The solution to this hurdle is the insurance company's attitude towards pharmacogenomics testing, increasing the evidence basis and proving that the testing costs will be saved and its influence will be beneficial [11]. From a medical insurance payment perspective, this requires the government to weigh the cost of genetic testing and the patient's medical expenses and patient benefits based on future research and data accumulation in health economics or pharmacogenomics [48]. In the recent years, some countries, such as the USA, China, and so on, have included genetic testing in national public health insurance. Table 15.1 shows the new insurance policy of individual countries on it. With the development of pharmacogenomics and personalized precision medicine, more and more pharmacogenomics tests will be included in reimbursement.

15.5 Conclusion and Prospect

With the support of the national government, key technologies related to the development of pharmacogenomics will be gradually improved. Pharmacogenomics will play an increasingly important part in the management of translational medicine and rational drug use. The idea of pharmacogenomics and individualized medicine will be accepted by more and more people and will gradually become the direction of modern medical development. It is believed that in the near future, the concept of personalized precision medicine will be deeply rooted in the hearts of the people and create a new chapter in the modern medical revolution in China, even more the world.

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