Genetics and Sports

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Preface

Athletic performance and the occurrence of sports-related injuries are both multifactorial conditions which are determined by the complex and poorly understood interactions of both environmental and genetic factors. Although much work has been done to identify the non-genetic components that are associated with performance and susceptibility to injuries, there is an ever growing body of research investigating the genetic contribution to these phenotypes. This book, which contains contributions by a broad range of scientist and clinicians from several disciplines – including, but not limited to, human molecular genetics, clinical genetics and exercise science – covers a number of topics in an attempt to obtain an integrated and holistic understanding of the field. The recent sequencing of the human genome and development of several tools and methodologies have successfully been used to investigate the genetic contribution to many complex diseases. This area of research has more recently been applied to the fields of exercise science and sports medicine. This publication reviews past, current and future applications, as well as the ethical concerns, of genetic research in the fields of exercise science and sports medicine.

The introductory chapter of this book highlights current and key concepts in human genetics, in particular with respect to its application to understanding multifactorial conditions. This is followed by a chapter exploring the often misunderstood relationship between nature (genetics) and nurture (common environmental effects such as training, diet, etc.) in determining athletic ability. The third chapter summarises the methodologies initially used during the pre-molecular biology era and the estimates obtained for gene and environmental contributions for performance-related phenotypes. The molecular genetics of performance is specifically investigated in the subsequent four chapters. These chapters include a review of the specific DNA sequence variants currently believed to be associated with athletic performance and response to training, critical reviews of the roles of the much investigated variants within the angiotensin-converting enzyme and α-actinin-3 genes and performance, and finally an exploration of the genetic and lifestyle of the East African runners who have dominated distance running events for the last half century.

This book also contains a chapter exploring the interaction of lifestyle, such as physical active or sedentary, with genetic make-up and its implications on human health, in particular understanding mechanisms underlying specific diseases, such as obesity, and its prevention. This is followed by a chapter summarising the recent developments in the identification of genetic risk factors for musculoskeletal soft tissue injuries.

The current and possible application of gene therapy as well as other novel therapeutic strategies such as stem cell and growth factor therapies in injured athletes is also reviewed. This is followed by a chapter exploring the potential abuse of genetic information and technologies, together with other developments in molecular biology, (gene doping) to enhance performance. The ethical framework in which genetic research is done and its application is not straightforward. Exercise scientists and sports physicians need to keep abreast of advances in medical ethics broadly and customise them efficiently to the field. These issues are discussed in the second last chapter, which is followed by a review on the new technologies and paradigms which will influence future genetic research in exercise science and sports medicine.

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Key Concepts in Human Genetics: Understanding the Complex Phenotype

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Abstract

The recent sequencing of a reference human genome has generated a large number of DNA-based tools, which are being used to locate genes that contribute to disease. These tools have also enabled studies of the genetics of non-disease traits such as athletic fitness. Sport scientists should keep in mind three major factors when designing such studies and interpreting the literature. First of all, the methods used to assign a biological trait (be it performance related or disease related) to a specific gene are not as powerful as is commonly believed. Second, the methods used are thought to be more robust for disease-related traits than for normal physical characteristics, likely because there are many more biological factors contributing to the latter. Third, additional levels of variability continue to be uncovered in the human genome; these may ultimately contribute more to physical differences between human beings than the levels studied over the past decade. This introductory chapter will aim to equip the reader with the necessary vocabulary to understand and interpret genetic studies targeted to sport fitness and sport-related injury.   Copyright © 2009 S. Karger AG, Basel

Key Genetic Concepts and Terms

Since the earliest days of competitive sport, athletes and their trainers have known that athletic performance is modifiable by dedicated training. However, some individuals appear to be naturally gifted with athletic ability, such that their performance prior to training is above average, and their performance after training is consistently excellent. Often encapsulated as the 'nature vs. nurture debate', the degree to which athletic ability is determined by inherited physical characteristics, as opposed to training-and diet-based regimens, has excited much interest.

The identification of genes that encode discrete biological molecules opened a new avenue of investigation – that of correlating physical traits with differences between individuals in DNA sequence. In many cases, it is easy to see how a physical trait such as height might impact on athletic performance: longer legs allow for greater distance

to be covered with each stride. Increased cardiac output or increased lung capacity (relative to overall body size) would similarly be predicted to enhance athletic performance. The rapid growth in tools for DNA analysis has allowed researchers to identify an increasingly diverse number of genes and genetic markers, and in turn to correlate these with physical characteristics.

The biological information necessary to form all tissues of the human body is contained in the fertilized egg. Most of this information is physically located in the nucleus of the cell, though some is contained within energy-processing mitochondria. As the fertilized egg divides, the DNA that carries this information is copied and redistributed throughout the new cells as they are made. The term gene is used to refer to a discrete sequence of DNA that produces a biologically active product. If different sequences of the same gene coexist in a population, that gene is said to have different forms termed alleles. An allele is a variant sequence of an identified gene. Persons with different alleles for a particular gene are said to be heterozygous, those with identical alleles on both chromosomes are homozygous. The genome is the sum total of all genes and connecting sequences present in an individual or a population. The term 'genome' can be used to refer to one unique series of genes (haploid genome), to the redundant series of genes present in an organism (diploid genome), or to the collection of genes present in a large number of individuals under study (population or species genome). Such distinctions are important, because it is often assumed that information from population studies may be directly applied back to individuals. This may or may not be the case, as will be discussed later.

DNA is made up of a combination of four chemical bases, referred to by the letters A, C, G and T. A string of such letters makes up a DNA sequence, such as AATGCG. By default, the most common combination of letters found in a particular region of the genome is taken to be the 'normal' sequence. Differences from that order of nucleotides are considered to be DNA variants. Many variants are possible. In the example above, the sequence AAGGCG would be considered a variant, because it differs at the third position from the left: a G exists where a T would normally be found. Variants that change one chemical letter are referred to as point mutations or single nucleotide polymorphisms (SNPs), depending on their frequency in the population and whether they have a major effect on health. If the AATGCG sequence were present in 55% of the sequences in a study population, and the AAGGCG sequence were present in 45%, the 'T' variant would be considered the 'normal variant,' and the 'G' variant would be considered a SNP. If, on the other hand, 99.95% of sequences studied were found to be AATGCG and 0.05% were found to be AAGGCG, the 'G' would be considered a rare variant and termed a 'mutation'. Debate exists whether the term 'mutation' should be reserved exclusively for when the 'mutant' DNA sequence has major effects on physical characteristics, or perhaps for when the variant can be proven to have arisen at a definable time. For rare disorders wherein a DNA sequence variant is 100% associated with disease, the use of 'mutation' is well-recognized. In human population studies wherein the function of the DNA sequence may not be clear, the

term 'mutation' is often used if the DNA variant is present only rarely in the population (i.e. at a frequency of 1% or less). In this chapter, no attempt will be made to settle this debate, and both the terms 'rare variant' and 'mutation' will be used, because both are found frequently in the literature.

The genotype of an individual refers to his or her specific DNA sequence for a known gene or DNA marker. By a similar derivation, the phenotype is a specific physical trait in an individual. Either term may be used narrowly to describe an individual sequence (e.g. AATGCG) or trait (e.g. total lung volume). Either term may also be used broadly, to refer to the sum total of all genetic or physical traits measured in a study. A haplotype (haploid genotype) refers to a known series of DNA sequences that occur on the same chromosome, and thus on the same strand of DNA. Haplotypes are often used in genetic studies, and may or may not contain specific genes. Haplotypes may contain a combination of SNPs, point mutations and other DNA sequences.

Mendelian traits are physical traits that obey Gregor Mendel's laws of inheritance, and hence are encoded by distinct genes. Genetic diseases are typically referred to as mendelian, but it is important to recognize that most physical traits associated with athletic performance will not be mendelian traits. Mendelian traits are transmitted with high frequency through families, which means that the risk to an immediate relative (parent, sibling or offspring) is rather high, on the order of 25–50%. Dominant traits are passed directly from parent to child; one allele is inherited from one parent who also has the trait, be it a healthy or a disease-related trait. Siblings and offspring of someone with a dominant trait have a 50% risk of inheriting the same allele, and hence the same trait. Recessive traits manifest in the children of two carrier parents; one mutant allele must be inherited from each (unaffected) parent, leading to a 25% risk to siblings. A special exception exists for sex-linked/X-linked recessive traits; these manifest in 50% of male siblings, with a 25% risk to all siblings when males and females are considered together. For mendelian traits, the physical trait correlates very highly with the susceptibility allele; often, 100% of individuals who inherit the mutant gene(s) will manifest the trait. It is also possible for an allele to be codominant, wherein heterozygotes manifest a phenotype that is intermediate between either of the two possible homozygote phenotypes. Tautological though it may be to say, complex traits are not so simple. Complex traits are physical characteristics that are influenced by both genetic and environmental factors such as diet, intrauterine environment and activity level. Complex traits are often quantitative traits, and in the normal population these terms are largely equivalent. Quantitative traits are physical characteristics for which a range of measurable values are possible, and are usually considered to be continuous variables. Quantitative traits include height, weight, forced vital capacity, stroke volume, etc. By contrast, qualitative traits are discrete physical characteristics that are either present or absent. Most complex traits encountered in sport science are assumed to be quantitative traits.

Genetic mapping is the process of assigning biological traits to genes, and/or of assigning genes to specific regions of chromosomes. Chromosomes are packages of

DNA that are visible during cell division. Because chromosomes are easily studied, they have become a convenient handle for gene mapping. Genes are mapped onto a chromosomal space much like a postal address might be noted on a cartographer's chart. A distinction should be made between genes (defined above) and DNA markers. DNA markers (also known as genetic markers or sequence tags) are used as reference points to describe the location of genes relative to each other. Just as a landmark on a map can be any feature of significance (a bridge, hill, church, pub, intersection, etc.), a DNA marker is merely a nucleotide sequence of known chromosomal location: it need not have an identifiable RNA or protein product. SNPs are a commonly used type of DNA marker, in part because a large number have been identified. SNPs are often assigned an 'rs' (reference SNP) number, such as rs1815739. It is easy to determine whether an individual carries a particular SNP, and to determine how many copies of that SNP he or she has. For the above reasons, SNPs are well suited for population studies. Apart from their use as markers, most SNPs do not have a known biological function.

A locus is a region of a chromosome that has a physical trait, DNA marker, allele or gene associated with it. A locus may be a theoretical entity and not necessarily a gene, though a genetic locus is often assumed to contain a gene with functional relevance to the physical trait under study. The locus itself may be a DNA sequence that controls the activity of one or more genes (e.g. by binding an activator protein). The locus can also be a 'neighbour' to a nearby sequence that is the actual determinant of the trait. If epidemiological data associate a physical trait such as height with a particular chromosome, that region is said to be a 'locus' for height [1]. It may be helpful to think of a locus as a low-resolution hypothesis: once data are sufficient to associate a physical trait with a region of the genome, a locus is defined and the relevant gene(s) or other sequences in the region are sought using higher-precision methods. A locus is a type of genetic 'neighbourhood', used as a term of reference until the actual 'address' is found in terms of the specific DNA sequence. Additional information, concepts and excellent diagrams may be found in the review by Attia et al. [2].

Genome-Wide and Candidate Gene Association Studies

The recent sequencing of the human genome has generated an extraordinary number of tools for DNA analysis, including several million SNPs [3]. Notable successes in the identification of rare mendelian single-gene disease traits have spurred widespread enthusiasm for DNA analysis as a predictive factor for disease risk. The rapid decline in the cost of genotyping these SNPs has spurred an equally rapid growth in studies that attempt to determine the contribution of genetic variation at the level of the DNA sequence to physical variation at the level of the whole organism. Analysis of such contributions is termed genotype-phenotype correlation. Though the usefulness of DNA analysis for mendelian genetic disorders is

undeniable, the usefulness of DNA analysis for most complex traits remains to be clarified.

An association study is an epidemiological tool that measures the degree to which an identifiable factor accounts for the risk for a particular disease. The factor may be exposure to an environmental agent (e.g. cigarette smoking) or 'exposure' to carrier status for a genetic trait (e.g. a DNA marker near the myoglobin gene) [3]. A genetic association study correlates an identifiable genetic trait with risk for disease or 'risk' for improved health. Typically, these studies either employ a candidate gene approach or a genome-wide approach. Studies that select a specific gene or genes and correlate variation in these genes with a disease or physical trait are termed candidate gene association studies. The genes in question are considered as candidates based on their known function, presumed to be relevant to the disease under study. Studies that examine DNA markers at many positions on multiple chromosomes are termed genome-wide association studies (GWAS). A GWAS will employ a large number of DNA markers that are scattered throughout all chromosomes. Each of these markers will be studied ('typed,' short for 'genotyped') in a population of individuals, and these study participants will also be assessed for their physical characteristics (i.e. their phenotype). The ultimate goal of both candidate gene and GWAS is to establish genotype-phenotype correlations. A genotype-phenotype correlation is an association between a DNA sequence and a physical trait. The ultimate association may be strong or weak. Single-gene mendelian traits are examples of strong genotype-phenotype correlations; a child who tests positive for two copies of the delta-F508 mutation in the *CFTR* gene is extremely likely to develop cystic fibrosis later in life [4], and specific mutations in human myostatin correlate strongly with increased muscle mass [5]. However, it is important to remember that most genotype-phenotype correlations for nondisease traits are rather weak. Red hair and green eyes are often seen in individuals who carry polymorphisms at the *MC1R* gene, but DNA variation at this locus does not guarantee that someone will develop these traits, nor is *MC1R* the only gene that controls hair and eye pigmentation [6].

Candidate gene association studies are less expensive than GWAS, because fewer markers must be genotyped. Thus, candidate gene studies are frequently performed to assess the risk of sport-related injury that might be imparted by a particular DNA variant [7]. A SNP in one of the collagen genes could be genotyped among swimmers with rotator cuff injuries and among swimmers without such injuries, to assess the risk conferred by carrier status for that SNP. Equally well, candidate gene studies could be performed to assess the 'risk' for high performance [8]. A SNP in or near the gene for hemoglobin might be genotyped in a number of endurance runners, and the frequency of this SNP among high-performance endurance runners (the 'cases') compared with members of the general population (the 'controls'). Several such studies have been done, but sport scientists should interpret reported findings with caution.

Though candidate gene studies are inexpensive and relatively straightforward to do, the initial associations that they discover are usually not found to be true when

studied in more detail. There are a number of reasons for this high false discovery rate (reviewed elsewhere [9]); it can be difficult to eliminate all possible sources of bias in a candidate gene association study. A major source of bias in many studies is population genetic substructure: the historical mixing between human populations that differed in the prevalences of genetic and physical characteristics. Suffice it to say that the actual ancestry of most individuals is much more complex than can be identified through questioning by a researcher, and this fact can lead to spurious statistical associations between ancient genetic markers and contemporary physical traits [10]. Other issues such as potential genotyping errors, blinded testing and reporting bias are also worth considering, but are not dealt with in detail here.

Definitive proof that a candidate gene (or variant in such a gene) is truly predictive of risk for sports injury would require a long-term prospective study in which DNA samples were collected in a large cohort of individuals, who were then followed over time until a sufficient number of them had developed the injury. Since this type of study is tremendously time consuming and expensive, an intermediate standard of proof is typically considered acceptable, wherein the findings of the preliminary association study are replicated in a larger population. For example, an association between SNPs in collagen genes and rotator cuff injury described in 100 cases and 250 controls from one academic center in a large city might be considered to be 'replicated' if, on assessment on a nationwide basis, carriers of the at-risk variant were found to have a higher prevalence of rotator cuff injuries in the previous 10 years than noncarriers. Typically, however, when such replication studies are attempted, the previous statistical association is no longer seen. Part of the difficulty likely lies in the fact that it is easy to come up with a biologically plausible hypothesis about how a particular DNA variant *might* influence disease. Whether the variants that *actually exist* in the human population *truly do* influence disease is another matter entirely. The answer is unknown at the time the study is done, but the accumulated experience from a number of candidate gene association studies for a wide variety of biological traits suggests that this method is prone to a high false discovery rate (i.e. the positive predictive value is low) [9, 11].

GWAS are thought to be more robust to replication than candidate gene studies. The former are often touted as being 'agnostic' or 'hypothesis-free,' since no prior assumptions are made about the specific gene or genes involved in susceptibility to disease (or high performance). However, GWAS are not, in fact, hypothesis-free. The hypothesis they test is whether there is biological variation in the human genome that is common enough to confer sufficient population-attributable risk to be detectable by the methods used. In simpler terms, GWAS test the hypothesis that there is, in fact, an association *somewhere in the genome*.[12] GWAS have achieved notable successes with biological traits for which genetic susceptibility is believed to be relatively high [13]. However, like candidate gene association studies, the loci identified in GWAS often are not replicated when larger studies are done, or when attempts are made to narrow the locus down to a specific gene [9]. In this context, it is particularly

important to remember that most genotype-phenotype correlations for nondisease traits are rather weak. A corollary to this is that, for most performance-related traits and for most sport-related injuries, evidence for genetic contributions should be weighed carefully and replicated in large independent populations before being accepted as valid [14]. Because of the large number of SNPs genotyped, GWAS perform a number of comparisons that is several orders of magnitude greater than most epidemiological studies; thus, it is appropriate to seek much lower p values (on the order of 5×10^{-8} instead of 0.05) [15, 16].

SNPs vs. Copy Number Variants: New Discoveries, New Technologies and New Challenges

GWAS also fail to assess all of the variability in the human genome. It has recently been discovered that a large amount of genetic differences between individuals are due neither to mutations nor to SNPs, but to duplications and deletions of genes or groups of genes [17, 18]. The groups of extra and/or missing DNA sequences on human chromosomes have been termed copy number variants (CNVs) [19], and reveal a previously uncharacterized architecture of the genome. Thus, the size of a normal individual's genome may differ substantially from that of other normal people, and from the theoretical 'reference human genome.' The regions that differ may contain entire genes or groups of genes, such that people may vary not only in their sequence at specific detectable SNPs, but also in the number of genes that they have. Most of these CNVs are transmitted stably through families and do not cause genetic disease. It may be helpful to think of the presence of CNVs as representing a certain amount of 'wobble' in the size of the human genome.

The global significance of CNVs for normal physiology is not clear, but CNVs are thought to hold significant potential as a source of phenotypic variation. Indeed, the impact of CNVs on human physical characteristics may well be greater than the impact of SNPs, either at the individual or population levels. This is a new and exciting area of research; whether CNVs impact athletic performance or susceptibility to sport-related injury remains to be evaluated. Estimates vary, but each person probably has approximately 12 of these CNVs (6 per haploid genome), and roughly half of the CNVs found so far encompass one or more genes [19]. The impact of a particular insertion or deletion CNV on physical characteristics depends on the gene(s) involved, which correlates with the size of the CNV and the gene density of the region where it occurs. At the time of this writing, nearly 20,000 human CNVs have been described, at just over 6,000 genomic sites (http://projects.tcag.ca/variation, accessed 16 Jan 2009). A relevant feature of these polymorphisms is that CNVs tend to be found outside of functioning genes rather than within them. The fact that gene-rich areas are relatively depleted of CNVs suggests that CNVs occurring in or near genes tend to have a mildly harmful effect on those genes. If CNVs had no such effect,

they would likely be distributed more randomly throughout the genome. The corollary of this phenomenon is that CNVs that are found in, near or including genes are expected to have a higher likelihood of having a biological effect than a SNP (possible exceptions include SNPs that result in an amino acid change in the protein product of a gene).

The Implications of Human Genomic Structure for Sport Science

Sport science researchers who are contemplating genetic studies that target traits such as high performance or injury susceptibility should be aware of the following properties of the human genome. The proportion of the genome that is made up of functioning genes is believed to be less than 3% [20]. The average coding length of a human gene is approximately 1,500 bp or 1.5 kb [21]. Estimates of the frequency of SNPs in the human genome vary from between 1 in every 180th nucleotide pair to 1 in every 279th nucleotide pair, on average [22, 23]. Thus, it is likely that polymorphic SNPs will be present in or near any candidate gene(s) selected for study. The proportion of the genome that is susceptible to copy number variation is currently believed to be on the order of 12% [24], an estimate that is likely to rise as more types of CNVs are described and better methods are applied to their detection. Thus, study designs should not focus exclusively on SNPs in plausible candidate genes – they should incorporate methods that assess copy number variation of the gene of interest. Similarly, GWAS should incorporate assessment of CNVs throughout the whole genome of the study participants. Copy number variants may be uncovered through the use of technologies like microarray comparative genomic hybridization ('array-CGH') and representational oligonucleotide microarray analysis (ROMA) and paired-end mapping, techniques that compare an individual's genome to a reference genome made up of an 'average' of several individuals' DNA [19, 25]. Several microarrays are available on the market [26]; these arrays have different levels of resolution, which translates into the ability to detect insertions and deletions of different sizes. Detection accuracy may also be improved by using multiple independent computational approaches to analysis of the data [27].

In addition to corroborating data from in vitro functional studies (such as expression levels for each identified allele), validation of any association findings will require replication in larger cohorts, ideally in one or more independent populations, and preferably with a prospective study design. Such stringent criteria are difficult to achieve, particularly among performance athletes where large sample sizes may not be available. At the very least, large control cohorts should be sampled from the same ethnocultural population as the athletes themselves, such that adequately narrow confidence intervals are available for the 'background' frequencies of the DNA variants studied. Population substructure also may dramatically skew the results of association studies [10]. Markers of population ancestry exist and should be genotyped where

possible, particularly in populations where admixture is known to have occurred historically [28].

One major strength that sport science may draw upon is the fact that numerous tools are available to characterize the phenotype of performance athletes in detail, and to define the precise nature of the pathology in injured athletes. Detailed phenotyping, properly applied, may improve the power to detect a biological effect in a small cohort (relative to a large cohort that is studied in less detail). Similarly, detailed genotyping that incorporates DNA markers of population ancestry and assessment of copy number variants should be the goal of studies designed to correlate performance traits with human genes.

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Nature versus Nurture in Determining Athletic Ability

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Abstract

This chapter provides an overview of the truism that both nature and nurture determine human athletic ability. The major thesis developed is that environmental effects work through the process of growth and development and interact with an individual's genetic background to produce a specific adult phenotype, i.e. an athletic or nonathletic phenotype. On the nature side (genetics), a brief historical review is provided with emphasis on several areas that are likely to command future attention including the rise of genome-wide association as a mapping strategy, the problem of false positives using association approaches, as well as the relatively unknown effects of gene-gene interaction (epistasis), gene-environment interaction, and genome structure on complex trait variance. On the nurture side (environment), common environmental effects such as training-level and sports nutrition are largely ignored in favor of developmental environmental effects that are channeled through growth and development processes. Developmental effects are difficult to distinguish from genetic effects as phenotypic plasticity in response to early life environmental perturbation can produce lasting effects into adulthood. In this regard, the fetal programming (FP) hypothesis is reviewed in some detail as FP provides an excellent example of how developmental effects work and also interact with genetics. In general, FP has well-documented effects on adult body composition and the risk for adult chronic disease, but there is emerging evidence that FP affects human athletic performance as well.   Copyright © 2009 S. Karger AG, Basel

Human athletic ability is determined by numerous sociocultural, psychological, anatomic, and physiological factors. The biological factors, such as an individual's aerobic capacity, are frequently termed complex traits, i.e. traits with a multifactorial genetic and environmental etiology. Gifted athletes are clearly a collection of many complex traits and so the athletic phenotype must arise from of a fortuitous combination of many genes and many environmental exposures. Some environmental effects are obvious and conceptually straightforward, like training. For example, it would be inconceivable to win the Tour de France without a years-long program of physical preparation. Other environmental effects, perhaps a majority, are indirect and

poorly understood. For example, it is unknown whether vigorous physical activity in childhood is a necessary precursor for the development of an elite athletic phenotype in adulthood [1, 2]. On the genetic side, major gene effects are also conceptually straightforward, but most genetic effects are small and/or indirect reflecting gene-gene interaction (epistasis) or gene-environment (GE) interaction. Interaction effects are frequently not trivial, as demonstrated in recent studies of epistasis and physical activity traits in mice [3]. Further, it should also be considered that many elite athletes are the product of GE correlation which occurs when an individual self-selects for, or is urged to participate in, a sporting discipline for which they show an early aptitude. Thus, a complete explanation to account for the full range of human variation in athletic performance remains a considerable challenge.

This chapter will take a broad view and will present evidence that both nature (genetics) and nurture (environment) determine the variance in human athletic ability. Because many of the other papers in this volume focus on specific genes and sport performance, this chapter will instead provide a historical perspective vis-à-vis genetic research with some emphasis on the limitations of current approaches and areas for future investigation. For environmental effects, this chapter will focus on 'developmental effects' defined as environmental effects that are channeled through the process of growth and development leading to irreversible changes on the adult phenotype. Developmental effects have emerged in the public health literature as an important causal mechanism with the power to explain some of the variance in adult chronic disease risk. Thus, the chapter will explore the thesis that such effects could also have a major impact on human athletic performance.

Genetics and Athletic Ability

Multiple lines of evidence going back more than one century leave little doubt that genes influence athletic ability [for reviews, see 4–10]. Quantitative genetic studies of twins, families, or extended pedigrees yield a population summary statistic termed *heritability* (h^2), where heritability in the *broad sense* refers to the proportion of phenotypic variance attributable to all genetic effects. Numerous studies [reviewed in 9] have demonstrated a significant h^2 for traits relevant to athletic performance. These include studies of aerobic capacity and the response to training [11–13], anaerobic performance [14, 15], muscle strength and power [16–18], neuromuscular coordination [19], bone density [20], body size and composition [21–26], muscle fiber type distributions [27–31], cardiovascular variables including blood pressure [32–37], blood lipid biochemistry [38], blood glucose, insulin, and peripheral insulin sensitivity [24, 39–41], substrate utilization and metabolic rate [42–44], pulmonary function [45–49], and hormones and hormonal response to training [50–52]. One frequently cited study is that by Bouchard et al. [11, 12] revealing significant familial aggregation for VO_{2max} and VO_{2max} trainability for individuals in the sedentary state, after

controlling for age, sex, body mass, and body composition. Heritability for both of these traits was near 50%, suggesting a substantial genetic contribution to baseline aerobic capacity and the response to training.

Unfortunately, heritability studies do not reveal the specific genetic architecture of a complex trait, i.e. these studies cannot: (1) distinguish between polygenic and/or major gene effects; (2) provide information on allele frequencies; (3) describe the mode of inheritance; (4) localize the chromosomal locations of genes, or (5) identify the gene products involved. To identify specific genes, two major approaches are widely applied: family-based linkage analysis and gene-association studies. Family-based linkage analysis is conducted on related individuals and the approach is based on the co-segregation of a putative (causal) trait locus with a known marker locus. Linkage implies the close proximity on the chromosome of causal and marker loci. Linkage analysis has a long history of success in mapping loci for simple monogenetic (mendelian) diseases such as Huntington's disease [53], but generally the approach only works if the causal locus has a high penetrance and a large effect on the trait in question. Linkage analysis may not have adequate statistical power and mapping resolution to detect genes of modest effect [54]. This is an important issue for understanding the variance in human athletic performance as elite athletes likely emerge on a favorable genetic background where individual alleles are both common in the general population and have only modest effects on the phenotype. In any case, there is a small literature reporting results of family-based linkage analyses with direct relevance to athletic performance. Numerous loci have been linked to traits which include VO_{2max} and VO_{2max} response to training [55–57], stroke volume, cardiac output, and blood pressure during exercise [58–62], training-induced changes in body composition [63–66], and insulin and glucose response to habitual physical activity or exercise [67].

Association studies include candidate gene approaches and various association mapping strategies that are conducted at the level of the whole genome. Candidate gene studies have proliferated in the last two decades since early studies in the 1970–1980s demonstrated no association of human physical performance traits with the classical blood group polymorphisms [9]. In a recent iteration of the Human Gene Map for Physical Performance Phenotypes [68], candidate gene studies comprised the majority (>90%) of the total literature. Interestingly, a sizable proportion of that literature (~15%) was focused on one specific gene polymorphism, the angiotensin converting enzyme (ACE) insertion/deletion polymorphism. Despite intensive focus, the significance of the ACE gene, in a genetic causal sense, remains controversial due to the inherent limitations of association studies. The candidate gene approach requires large sample sizes to identify loci with small effects. Additionally, the approach is susceptible to the problem of false association due to chance, bias, or confounding [69]. This is true both for case-control studies, i.e. comparing allele frequencies in elite athletes with the general population, and in studies that evaluate mean phenotypic values by genotype in a homogenous study group.

Genome-wide association (GWA) strategies rely on linkage disequilibrium to detect associations between traits and gene markers. GWA is a powerful and efficient means to dissect the genetic basis of complex traits [54, 70], and the advent of high-density genotyping arrays has allowed a shift away from candidate gene studies to GWA. Using GWA, there have been many recent successes in the elucidation of genes involved in disease processes such as type 2 diabetes [71–75], breast cancer [76–80], and prostate cancer [81–85]. Two key elements of these successes have been the collection of large sample sizes (i.e. thousands of individuals) with the consequent increase in study power to detect loci of modest effect, and the exponential advances in genotyping technologies that have dramatically improved genome coverage. To date, GWA has not been applied explicitly to study exercise traits, although some traits, such as body composition, may be relevant to human performance. Like candidate gene studies, GWA can be hindered by population stratification that can lead to an increase in false positives [86–89].

Another potential complicating issue that future genetic studies of human performance will need to consider is that there is extensive structural variation in the human genome [90–95]. Here, the term structural variation broadly refers to genomic alterations involving DNA segments larger than 1 kb [94, 96]. This term includes balanced changes (e.g. inversions, translocations) and those that alter copy number (copy number variants or CNVs). Of particular interest is the large number of CNVs described in humans. As of September 2008, more than 5,000 CNV loci have been catalogued in the Database of Genomic Variants (available at http://projects.tcag.ca/variation/). Not surprisingly, there have been recent studies investigating the role of CNVs in human phenotypic variation. In addition to the well-known influence of CNVs on sporadic and mendelian diseases [96, 97], recent research has indicated that CNVs have an important impact on levels of gene expression [98], and also play a role in complex traits and diseases, such as Crohn's disease [99], systemic lupus erythematosus [100], psoriasis [101], autism [102, 103], and schizophrenia [104]. Interestingly, there is also evidence indicating that CNVs may be involved in adaptation to different environments. A recent study has shown that individuals from populations with high-starch diets have, on average, more copies of the salivary amylase (AMY1) gene than those with traditionally low-starch diets [105]. Salivary amylase is responsible for starch hydrolysis, and AMY1 copy number is correlated positively with salivary amylase protein level. It has been proposed that having higher AMY1 copy numbers improves the digestion of starchy foods and for this reason AMY1 copy number has been subject to natural selection in human populations. As a working hypothesis, CNVs could affect human athletic performance particularly if variation exists in pathways that are directly involved in exercise, e.g. ATP synthesis, modulators of the cardiovascular response to exercise like ACE, and so forth.

As mentioned above, recent GWA efforts have identified hundreds of associations between common single nucleotide polymorphisms and complex traits and diseases [reviewed in 106]. However, it is important to note that the variants identified to date

only explain a small portion of the genetic risk. This is due to several factors: (1) the limited power of GWA studies to identify common variants with very small effects (e.g. those increasing disease risk by a factor of 1.1 or less) [106]; (2) the limited ability of previous commercial microarray genotyping platforms to identify structural variation that may increase disease risk [107], and (3) the lack of power of GWA studies to identify rare casual variants [108]. However, our understanding of the genetic architecture of complex traits and diseases will dramatically improve in the near future. The application of GWA approaches to increasingly larger cohorts will make it possible to identify common variants of small effect that have not been detected in previous genome-wide scans. The development of better maps of structural variation in the human genome and improved microarray technologies (such as hybrid microarrays containing single nucleotide polymorphism and CNV probes as well as higher resolution genome comparative hybridization arrays) will clarify the relative role of structural variation in complex traits. Finally, the impressive advances in next generation sequencing technologies [109] are already opening the door to large-scale re-sequencing studies, which will dramatically expand our understanding of the genetics of complex traits and diseases, including the role of rare variants.

The coming years will undoubtedly reveal many additional candidate genes and genomic mechanisms underlying human performance. However, individual genes and specific mechanisms will still probably only explain a small portion of the variance for a particular athletic trait. For example, in our own work with the ACE gene, we detected a highly significant association of the ACE I-allele with higher arterial saturation (SaO_2) during exercise at high altitude ($p < 0.01$) [110]. The ACE gene itself explained only about 4% of the total variance in SaO_2 compared with the effect of acclimatization (explaining nearly 44% of the variance). The remaining unexplained variance was attributable to measurement error, other uninvestigated genetic, genomic, and environmental effects, epistasis, and GE interaction. Again, the interaction effects may be substantial. For example, Hagberg et al. [111] detected a significant association of the ACE genotype with exercise systolic blood pressure, but the association was evident only in untrained versus trained women. In other words, the ACE gene effect in that study was a function of the training environment and it was detectable only because the authors took training into account in their study design.

Most environmental effects are poorly characterized and thus difficult to account for in genetic study designs, particularly those that act over the developmental period. Consider a study by Kajantie et al. [112] who measured oral glucose tolerance and ACE I/D genotype in 423 adult women. The I-allele was associated with higher insulin response, but this association was conditioned by birth weight, i.e. it was only significant in subjects of low birthweight (p for interaction = 0.003). Low birthweight was taken as a proxy for suboptimal intrauterine growth due to poor fetal nutrition. Thus, the study suggests that an early-life environmental experience (i.e. poor fetal nutrition) conditions the expression of genetic effects on the adult phenotype many years later. Conceptually, this is a clear example of GE interaction, but it is important

to emphasize that the interaction occurs across developmental time. The athletic phenotype probably emerges in much the same way, i.e. environmental effects work through and influence the trajectory of an individual's growth and development program while simultaneously interacting with an individual's genetic background to irreversibly affect the adult phenotype.

The Environment and Athletic Ability

Environmental effects begin as early as preconception via gametic imprinting [113]. Gametic imprinting is achieved through methylation of DNA during gametogenesis [114] a process which modifies parental gene expression resulting in parental-dependent traits in offspring. As an example, Cooney et al. [115] showed that maternal dietary manipulation in mice increased DNA methylation and affected offspring health and longevity phenotypes. It is not currently known whether important complex traits with relevance to athletic performance have significant epigenetic components, but a number of studies suggest that this is the case for some cancers and for chronic disease traits [116, 117].

Environmental effects continue after conception during the growth and development period. Developmental effects arise because many organ systems show plasticity based on environmental exposures during 'sensitive periods' of rapid growth including the gestational period, infancy, childhood, and adolescence. One of the best-documented examples of this phenomenon is the extreme plasticity of the respiratory control system. Neonatal exposures to hypoxia and hyperoxia result in permanently altered adult ventilatory responses [118, 119]. Another good example is developmental lung growth in response to early-life exposure to high altitude. Peruvians born at altitude have a nearly 10% larger forced vital capacity compared to genetically matched Peruvians born at sea level [120]. In dogs, this developmental lung response leads to an increase in the diffusion capacity for oxygen [121].

The prenatal period has received the greatest research attention based on the fetal programming (FP) hypothesis. The FP hypothesis posits that intrauterine nutritional stress produces small/thin babies at birth and results in a permanent re-programming of the fetal metabolic and hormonal milieu with long-term consequences for adult chronic disease risk (fig. 1). In support of the FP hypothesis, epidemiological studies show a strong association between low birthweight (again, as a proxy for poor fetal nutrition) and various chronic disease traits including the adult prevalence of obesity, type 2 diabetes, insulin resistance, hypertension, and cardiovascular diseases [122–127]. For example, in one study the prevalence of impaired glucose tolerance or diabetes fell from 27% in subjects who weighed 2.50 kg or less at birth to 6% in those who weighed more than 3.41 kg (p < 0.002 after adjusting for BMI) [125]. Interestingly, FP effects are graded and operate in a dose-response manner across the entire range of human birthweight variation, not simply below a lower critical threshold birthweight [126–128]. Also, a number of studies suggest that FP effects are strongest for low-
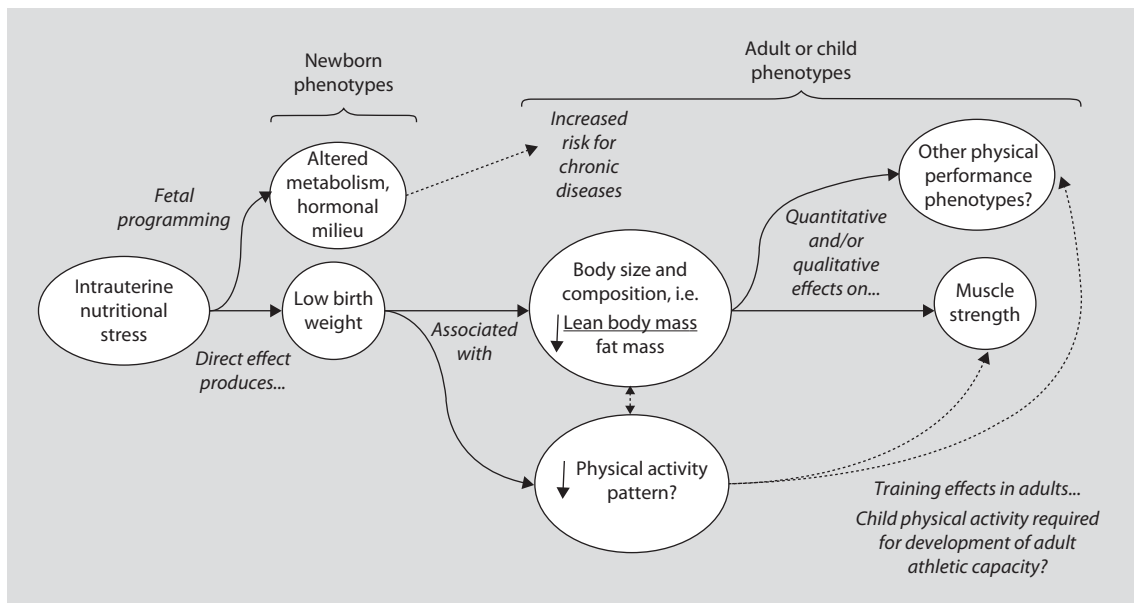
Fig. 1. FP and athletic performance conceptual framework. Fetal nutritional stress results in low birthweight and reprograms fetal metabolism with phenotypic effects into adulthood. Low birthweight is strongly associated with increased adiposity and reduced lean body mass (including muscle) in adults. Low birthweight may also be associated with decreased physical activity. These effects may account for decreases in muscle strength and other athletic performance phenotypes.

birthweight infants who then experience rapid postnatal increases in weight-for-age percentiles during the first few years of life, i.e. catch-up growth [129–131].

Figure 1 is given as a general conceptual framework for FP effects on human performance, and there is strong evidence for several of the causal pathways implied. Altered metabolism due to FP likely explains the strong association of low birthweight with increased adult adiposity and decrements in lean body mass (LBM) [132–134]. In other words, small-for-gestational-age babies tend to grow up relatively fatter and at the expense of lean tissue compared to normal-birthweight babies. The quantitative decrements in muscle mass account for the association of low birthweight with reduced grip strength, but there is also evidence of a qualitative effect of FP on muscle function/performance [135–137]. In addition, several recent animal studies show an association of low birthweight with decreases in voluntary physical activity in adult rats [138, 139]. If true, this would provide an additional pathway through which FP could affect adult body composition and/or muscle strength. In fact, lifelong physical activity/inactivity is a potential mediator between early life environmental effects and any number of performance traits from muscle strength to whole body aerobic capacity. In children this may be particularly important as adequate physical activity may be necessary for the normal development of muscle, neurological, and

cardiopulmonary systems [1]. The specific literature in support of this conceptual framework is reviewed in greater detail below.

Low Birthweight Predicts a Decrease in the Relative Lean Body Mass or Muscle Mass in Adulthood

Numerous studies show significant positive associations of birthweight with the absolute LBM, the fat-free mass (FFM), and/or the muscle mass, i.e. bigger babies grow up to be bigger adults. Therefore, the inference that FP causes a reduction in the relative amount of lean tissue requires adjustment for adult body size and/or a demonstration of commensurate increases in adiposity. Several studies of infants [140], children/adolescents [141, 142], and adults [132–134, 143–147] meet one or both of these criteria. For example the study of Singhal et al. [142] on 78 adolescent girls, which assessed body composition via bioelectrical impedance (BIA), showed that low birthweight was associated with lower FFM even after adjusting for adult height. Similarly, two BIA studies in adults showed that low birthweight predicts low FFM or LBM after adjusting for adult BMI or adult height [143, 147]. Two dual energy X-ray absorptiometry (DXA) studies in adults showed that low birthweight predicted low muscle mass, controlling for adult height [144], or that low birthweight predicted lower muscle mass commensurate with increased fat mass and percentage body fat [145]. Several other studies in this area are worth highlighting. The study by Kahn et al. [132] showed a strong association of birthweight with adult BMI. However, after adjusting for thigh muscle and bone area (assessed via anthropometrics) the strength of association dropped by 68%, as compared to a drop of only 30% when thigh fat area was used as a control variable. Thus, the authors concluded that the positive association of birthweight with BMI reflects increases in lean tissue as opposed to fat. In the study by Phillips [146] low birthweight was associated with smaller stature and body weight in adults, but much of the weight reduction was accounted for by a reduction in muscle mass as estimated by urinary creatinine excretion. Finally, studies by Loos et al. [133, 134] using a monozygotic twin approach showed that in young adult males and females, the heavier twin was taller and heavier as an adult, but when adjusted for body mass, heavier twins had greater LBM. Because monozygotic twins have the same genome and share certain aspects of the intrauterine environment, pair-wise twin comparisons allow for the control of genetic confounding factors and some potential maternal confounding factors. This strengthens the hypothesis that the amount of lean tissue in adulthood traces back to the twin-specific intrauterine experience.

Low Birthweight Predicts Increased Adiposity in Adulthood

The literature supporting increased adiposity in adults born with low birthweight is somewhat inconsistent. This may be due to the fact that the fat mass is more variable and subject to environmental effects in adulthood than the FFM. Also, increased adiposity is associated with both ends of the birth weight distribution, i.e. high birth weight is also associated with an increased prevalence of obesity in adults [148]. At

least seven studies of adults and children using skin folds only to assess body composition show a negative association of birthweight with relative body fat [133, 134, 141, 149–152], but at least two others show no association or a weak association in one or more study groups [141, 153]. Despite the limitations of the skin fold technique, the method does have the advantage that fat patterning can be evaluated. Among the studies cited above where fat patterning was described, there is an apparent consensus that low birthweight is associated with increased truncal fat in adulthood as opposed to limb fat [133, 134, 141, 150–152]. A number of studies have employed more direct methods of body composition assessment. Using BIA, Eriksson et al. [143] showed that low birthweight was associated with an increased fat mass controlling for BMI in adults. However, Singhal et al. [142] failed to find a similar association in adolescent girls controlling for height. Using DXA, a case control study by Kensara et al. [145] showed that low-birthweight subjects (n = 32) had a significantly higher percent body fat than controls. However, two other DXA studies in much larger samples showed no association of low birthweight with increased body fat or body fat percentage [144, 154]. To date, the most methodologically comprehensive study in this area was that by Elia et al. [155] who measured body composition using a four-component model that included skin folds, DXA, air densitometry, and deuterium dilution to measure total body water in 85 children (6–9 years). Results were consistent across body composition measurement techniques and revealed birthweight to be a strong predictor of percentage body fat in prepubertal children, i.e. an increase in birthweight of 1 standard deviation was associated with a decrease of 1.95% body fat. Only one study, in adults, employed the 'gold-standard' technique of hydrodensitometry [154]. In that study, there was no association of birthweight with fat mass or percentage body fat in a sample of 231 nondiabetic Pima Indians. However, given the high prevalence of diabetes among adult Pima, the study sample may not have been representative of putative FP effects that take place in the general population.

Low Birthweight Predicts Decreased Bone Mass and/or Bone Mineral Density in Adulthood

There is also strong evidence that the development of bone is modified in utero, a topic that has recently been reviewed [135, 156]. For example, an epidemiological study by Gale et al. [144] in a cohort of 70- to 75-year-olds (n = 143) showed significant positive associations of birthweight with whole body bone mineral content after adjustment for age, sex, adult height, smoking, alcohol consumption, calcium intake, and physical activity. Similarly, the study of Labayen et al. [141] showed a significant positive association of birthweight with bone mass adjusting for height in adolescent girls, but not boys. Studies of infants suggest that these effects are apparent at birth. Lappillone et al. [140] showed significantly lower bone mineral content in small-for-gestational-age infants compared to appropriate-for-gestational-age controls. Also, Godfrey et al. [157] showed that newborn birthweight is positively associated with bone mass after adjusting for sex and gestational age.

Low Birthweight Predicts Decreased Muscle Strength in Adulthood

Birthweight is positively associated with grip strength, a measure that reflects the action/strength of forearm flexors. Because of the positive association of birthweight with muscle mass (see above), and because muscle force depends on muscle cross-sectional area, FP effects on muscle strength must be largely mediated by changes in LBM [158]. However, there is also some indication that FP affects muscle quality/muscle performance. Three large-scale epidemiological studies of middle-aged and elderly subjects by the same research group in the UK show strong positive associations of birthweight with grip strength even after adjustment for various adult body size covariates [135–137]. For example, the study by Kuh et al. [137] showed a positive association of birthweight with grip strength, after adjusting for age, sex, and adult height. The effect was present in both men and women, and a 1-kg difference in birthweight in the cohort meant going from the 10th to 80th percentile of the grip strength distribution. While these results certainly imply effects of FP on muscle function, no study to date has adjusted for a direct measure of muscle mass. Further, Maltin et al. [159] have reviewed evidence of FP effects on muscle fiber number, fiber type, and fiber size. Apart from severe manipulations such as maternal starvation [160, 161], there is little evidence that FP has effects on muscle histology, including fiber type proportions. Thus, more work is necessary in this area.

Does Low Birthweight Predict Other Performance Phenotypes?

Taylor et al. [162] showed an association of impaired fetal growth (low ponderal index) with muscle performance in adult women as assessed by ^{31}P magnetic resonance spectroscopy. Low ponderal index at birth was associated with biochemical differences in adults that were consistent with delayed activation of glycolysis/glycogenolysis at the commencement of hard exercise stressing the anaerobic system. Similarly, a near-infrared spectroscopy study by Thompson et al. [163] showed no association of ponderal index with muscle histology, but differences in muscle reoxygenation rate. These authors suggested that the reoxygenation rate differences by ponderal index were consistent with the delay in the activation of anaerobic glycolytic metabolism demonstrated by Taylor et al. [162].

Does Low Birthweight Predict Physical Activity/Inactivity or Components of Energy Expenditure?

FP effects on body composition may be mediated through aspects of lifelong energy balance and/or physical activity/inactivity. Recent animal research suggests that FP operates directly on behavioral traits including the caloric intake and the voluntary physical activity. In rats, maternal undernutrition during pregnancy acts *directly* to produce offspring that are less physically active and also hyperphagic throughout postnatal life [138, 139]. This is intriguing because it suggests that associations between obesity, the metabolic syndrome, cardiovascular diseases, sedentary behavior, and overeating may have a common biological cause that traces back to intrauterine

experience. In the context of athletic performance, physical activity pattern is obviously directly associated with many performance measures, but serious consideration should also be given to the potential role of child physical activity pattern as a determinant of adult performance capacity.

Two human studies have examined FP effects on components of energy expenditure and/or energy metabolism [154, 164]. One study of 449 middle-aged men and women reported a higher resting pulse rate in low-birthweight subjects equivalent to about 2.7 beats/min (decline) per kg birthweight increase [164]. The authors suggested that this was due to elevated sympathetic nervous system activity that was established in utero. However, an equally plausible explanation is that low-birthweight subjects are less physically active, thus not aerobically fit, and therefore have lower stroke volume and a higher resting pulse rate. The other study in 272 Pima Indians assessed muscle sympathetic nervous system activity directly using microneurography and found no association with birthweight [154]. This study also measured the 24-hour energy expenditure, the sleeping metabolic rate (SMR), and the 24-hour respiratory quotient in a metabolic chamber. Of these measures, only the SMR was significantly associated with birthweight ($r = -0.13$), i.e. SMR was marginally higher than predicted for body size and composition (by ~2–3%) in the lowest versus highest birthweight subjects. The study did not measure resting energy expenditure or basal metabolic rate, and subjects were inactive during their 24-hour stay in the metabolic chamber. It should be emphasized that SMR is not a proxy for basal metabolic rate, and the association detected could reflect sleep disturbances in low-birthweight individuals. Thus, to date there is little direct information available on links between birthweight and components of energy expenditure in humans.

Conclusions

The genomic information revolution has made it possible to interrogate in new and powerful ways the genetic basis of human athletic performance. There is no doubt that this area of inquiry will become a major focus within the exercise sciences. However, it is also true that the genetic blueprint of an individual operates under the influence of innumerable environmental effects, and many of these effects operate at the earliest stages of the life cycle. In recent years, there has been an equally important revolution in developmental biology with the recognition that early-life environmental experience has lasting effects on the adult phenotype. This chapter has reviewed FP as an example of a developmental effect, but similar developmental effects may operate during infancy, childhood, and/or adolescence. Unfortunately, there is a dearth of information on these later time periods with direct relevance to adult exercise capacity. In any case, whenever developmental effects act, there is evidence that these effects interact with an individual's genetic background to produce irreversible change. If true, then accounting for developmental effects will be a key not only for

detecting genetic effects in future research but also for understanding the full range of variation in human athletic ability.

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Genetics and Sports: An Overview of the Pre-Molecular Biology Era

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Abstract

Estimated genetic and environmental contributions to individual differences in physical performance phenotypes, responsiveness to intermittent, aerobic and strength training, and specific skill training protocols are the focus of this chapter. Data are derived primarily from twin and family studies, although methods of analysis vary considerably. Estimates of heritability span a wide range for several performance phenotypes and the responsiveness to training. This is explained, in part, by differences in sample characteristics and analytical strategies. Corresponding data for skill acquisition are very limited. Data dealing with the effects of age, sex, maturation and ethnicity on heritability estimates are lacking, and information on behavioral phenotypes that may be related to performance is not available.   Copyright © 2009 S. Karger AG, Basel

Performance phenotypes, such as muscular strength and power, aerobic performance and motor performance are essentially outcome variables based on tests and measurements conducted under standardized conditions. The process of performance is not ordinarily considered. Hence, it is difficult to make inferences about sport performances which are carried out under dynamic conditions far removed from those under which performance phenotypes are measured. Allowing for this caveat, this chapter considers genetic contributions to individual differences in physical performance. Research methodology and estimates of genetic and environmental contributions to variation in performance-related phenotypes are briefly considered. Genetic and environmental contributions to several performance phenotypes are then evaluated followed by a brief consideration of responsiveness of several performance phenotypes to training and genetic considerations in skill acquisition. Finally, the pre-molecular biology methodology is placed within the past, present and future contexts for the study of genetic epidemiology in human performance.

Methodological Considerations

Physical performances and related factors are evaluated on a continuous scale of measurement. Distributions in the general population are Gaussian or skewed, which is typical for quantitative, multifactorial phenotypes that are influenced by multiple genes (polygenic) and environmental factors. As such, the issue is not a question of 'nature' versus 'nurture' [1]. Both nature and nurture and their interactions are important to understanding performance phenotypes.

Two basic approaches have been used to study the genetic basis of performance phenotypes and related characteristics: the unmeasured genotype approach (top down) – the focus of this chapter, and the measured genotype approach (bottom up) – the focus of other chapters [1, 2]. When the measured genotype is not available, inferences about genetic influences on a phenotype are based on statistical analyses of the distributions of measures in related individuals and families based on the theoretical framework of biometrical genetics [3]. Two major strategies are used: twin studies and family studies. The former includes both monozygotic (MZ) and dizygotic (DZ) twins. Since MZ twins have an identical genetic background, they will be more similar (higher intra-pair correlation) in a trait that is under genetic control than DZ twins who share on average one-half of their genes. With twin data, genetic and environmental factors unique to the individual and shared within families can be estimated and under certain assumptions dominant genetic effects can also be identified. In family studies, the similarities among parents and offspring and among siblings are studied, although generational differences imply both age-specific genetic and environmental effects, which increase complexity of analysis. The family approach permits the identification of genetic plus cultural transmission of traits and estimates of maximum heritability. If data from more extended or combined pedigrees are available, more sophisticated models can be tested.

Assessment of heritability is based on the model that total variation (V_{tot}) in a phenotype is partitioned into genetic (V_G), common environmental (V_C) and individual-specific environmental (V_E) components ($V_{tot} = V_G + V_C + V_E$). Heritability ($h^2$) refers to the proportion of the total variation that can be attributed to genetic effects (V_G/V_{tot}). It is generally assumed that the effects of different genes are additive (a^2) meaning that the genotypic effect of the heterozygote genotype on the phenotype falls exactly between the genotypic effects of both homozygote genotypes. Dominance genetic effects refer to the interaction between alleles at the same locus (heterozygote effect does not fall exactly in the middle between the two homozygote genotype effects) and epistasis describes the interaction between alleles at different loci. The contribution of environmental factors shared by family members (common environmental factors, $c^2 = V_C/V_{tot}$) and the proportion of environmental factors that act on an individual can also be estimated ($e^2 = V_E/V_{tot}$).

Several assumptions should be met when using the additive model: no interaction between gene action and environment (different genotypes all react equally to

similar environmental factors), no gene × environment correlation (similar exposure of environments for different genotypes), no gene × gene interaction, and finally no assortative mating for the trait studied (one assumes people mate randomly for the phenotype in question). In all likelihood, influences on performance phenotypes do not follow all assumptions, and gene action/environmental influences are more or less important at different ages, in each sex, in specific ethnic populations and/or in affluent versus developing countries. Longitudinal (transmission) models are needed to study age-specific influences on the decomposition of inter-age correlations within MZ and DZ pairs into genetically and environmentally transmitted or time-specific sources of variance [4–6].

The effects of gene × environment interaction in the responsiveness of individuals to physical training and specific skill training protocols is an important effect that is largely unstudied. Specific designs can be used to study this interaction. A set of MZ twins with similar baseline performance levels can be exposed to training regimen at a similar load and followed longitudinally. 'Repeated-measures' ANOVA can be used to quantify the variability of responses *between* different 'genetic pairs' versus the variability of training responses *within* the same 'genetic pair'. A significant F-ratio represents a significant genotype × training interaction, or genotype-dependent response to the training protocol [7]. Alternatively a longitudinal analysis of trained MZ and DZ twins can specifically test for the presence of 'training-specific' gene sets that are independent of gene sets that contribute to pre-training levels of performance [8]. A third alternative which permits the study of gene × environment interaction in twin studies without an intervention protocol is the inclusion of a measured environmental variable (e.g. hours of training per week) in the structural equation model [9]. This approach results in heritability estimates that vary with different levels of the measured environmental variable if gene × environment interaction is in fact present. The method requires large sample sizes and has not yet been applied with physical performance phenotypes.

Related Phenotypes

Body size, physique, proportions and composition are associated with performance in the general population and elite athletes. Among youth, individual differences in the timing and tempo of biological maturation are an additional source of variation [10, 11]. Although there have been considerable advances in behavioral genetics, behavioral phenotypes that may be associated with performance are not ordinarily considered in genetic analyses of performance phenotypes.

Evidence from family, sibling and twin studies indicates that a significant proportion of the variance in anthropometric characteristics (skeletal lengths, skeletal breadths, limb circumferences, bone mass) is genetically determined [2]. Heritabilities for stature from 19 samples of twins ranged from 0.69 to 0.96, with a mean and

standard deviation of 0.85 ± 0.07. A corresponding estimate for segment lengths was similar, 0.84 ± 0.10, while those for skeletal breadths and limb circumferences were more variable and lower. The preceding estimates are based largely on data from well-nourished samples of European (White) ancestry, and the genetic contribution to the variance in adult stature tends to be higher in these populations [11].

Body mass is under less genetic control than stature, but when corrected for stature the genetic determination of mass is about 0.40. Heritabilities of the BMI (weight/height^2) in MZ twins reared apart ranged between 0.40 and 0.70, while estimates based on adoption and large family studies were lower for percentage body fat and skinfolds [12]. Other studies, however, have reported higher genetic contributions to indicators of body fatness, for example 0.70–0.94 for the BMI, waist hip ratio, sum of skinfolds and 'bio-electrical impedance' – estimates of fat mass based on structural equation modeling in a large twin cohort [13]. Estimates from several Canadian studies indicated a maximal heritability of 0.40 for various indicators of body fat distribution [2], while estimates based on a ratio of trunk to extremity skinfolds in a longitudinal study of Belgian twins ranged from 0.78 to 0.88 [6]. Differences in reported heritabilities stem in large part from adjustments that are made or not made in the respective analyses. Estimated heritabilities tend to be lower when indicators of fatness were adjusted for size and when indicators of fat distribution were adjusted for size and fat mass.

Physique is most often assessed with the Heath-Carter anthropometric protocol for estimating somatotype [14]. Heritability estimates from twin studies, based mainly on adolescents, are generally >0.70 for the three somatotype components with few exceptions in late adolescent samples [15]. Estimates from family studies tend to cluster around 0.40–0.55 in adolescents and adults. In a large sample of young adult twin pairs, sex-specific heritabilities were 0.28, 0.86 and 0.76 for endomorphy, mesomorphy and ectomorphy, respectively, in males; corresponding heritabilities for the three components were, respectively, 0.32, 0.82 and 0.70 in females [16]. More than 70% of the total variation in each somatotype component was explained by sources of variance shared between the three components in adult men and women, reflecting the need to analyze the heritabilities of somatotype components with a multivariate approach [16]. The results also indicate that the three components of somatotype estimated with the Heath-Carter anthropometric protocol are to a large extent under the control of an identical set of genes and identical environmental factors. This also explains the high intercorrelations among components [4–6, 17]. The high interrelationships among somatotype components are a major shortcoming of the Heath-Carter anthropometric method. This is related, in part, to the lack of trunk measurements, especially in the derivation of mesomorphy, and use of the same measurements in deriving each component. Skinfolds and height are used to derive endomorphy; limb circumferences corrected for skinfold thickness, limb skeletal breadths and height are used to derive mesomorphy; and height and weight are used to derive ectomorphy.

Variability in measures of biological maturation in children and adolescents also has a significant genetic component. Major characteristics of the adolescent growth

Table 1. Heritabilities of muscular strength and power

	Twin studies	Family/sib-pair studies
Static strength	0.14–0.83 20 studies [4, 19–21, 23, 29]	0.27–0.58 5 studies [19]
Dynamic strength (isokinetic concentric and excentric)	0.29–0.90 3 studies [25, 26, 29]	0.42–0.87 (concentric only) 2 studies [27, 28]
Explosive strength or power (jump tests, Wingate test)	0.34–0.97 7 (recent) studies [5, 20, 23, 26, 31–34]	0.22–0.68 [31]

Heritabilities include both commonly used heritability indexes as well as heritabilities estimated based on structural equation modelling.

spurt – age at take-off, age at peak height velocity (PHV), height and height velocity at these time points, have heritabilities which range between 0.89 and 0.96 in both sexes [18]. Other indicators of biological maturity status – stages of sexual maturity, age at menarche, skeletal age and percentage of adult stature attained at a given age – all show relatively strong genetic influence [11].

Muscular Strength and Power

Genetic contributions to muscular strength and muscle power have been recently summarized [19]. Estimated h^2 in five family studies varied between 0.27 and 0.58 for static strength of different muscle groups and with adjustment for covariates such as sex, age and sometimes body mass. Estimates tended to be higher in twin studies, although the range of h^2 in 15 studies, 0.14–0.83, was greater than in family studies (table 1). Apart from limitations related to sample sizes and methodology used to obtain heritabilities, it should be noted that these estimates are based on samples that span childhood through adolescence into old age. While adjusting for age and sex is essential in family studies, many twin studies encompass broad age ranges without correction for age and do not always test for potential sex differences. Age-corrected heritabilities overlook the fact that genetic and environmental influences can change with age per se and also with biological maturation as genes can be switched on or off during childhood, adolescence and adulthood. Moreover, individuals differ in the timing and tempo of maturation and of aging. They also are exposed to different environmental influences during the life cycle. The use of same-aged twins eliminates the need for age correction, although it may be necessary to consider individual differences in the timing and tempo of biological maturation rather than chronological age [4].

More recent twin studies based on structural equation modeling are consistent in showing no evidence of a shared (familial) environment effect for static strength in adolescents of both sexes [4, 20], young adult men [21, 22], and elderly women [23]. This observation is credible since multivariate analyses increase the power to detect shared environmental influences. The heritabilities in Belgian adolescents ranged from 0.52 to 0.82 for boys (from 1 year before PHV until 3 years after PHV) and were somewhat lower in girls, 0.22–0.75 with one outlier of 0.07 [4]. The h^2 at 3 years after PHV, which approximates young adulthood, of 0.52 in boys and 0.48 in girls were consistent with those for young adult Swedish, 0.50 and 0.60 [22], and Belgian, 0.70 [21] men, and for elderly women, 0.49 [23]. These estimates based on similar analytical techniques suggest that the heritability for static strength may be somewhat higher during adolescence than in young adulthood and old age. Systematic analyses of sex differences in static strength in adulthood are limited with one study observing no sex differences in the relative contribution of genes and environment in a large sample of Danish twins aged 45–96 years, $h^2 = 0.52$ [24].

For maximal dynamic strength as measured by isokinetic dynamometry there is a paucity of data since the protocol is more impractical in large field-studies (table 1). Heritabilities of eccentric elbow flexor strength in 42 pairs of young adult male twins ranged from 0.62 to 0.82, which were larger than estimates for concentric flexion, 0.29–0.65 [25]. Heritabilities tended to be lower at higher contraction velocities. More recently, an opposite trend was noted in 15 pairs of female adolescent twins selected for similar environmental backgrounds and levels of habitual physical activity; heritability estimates increased at higher speeds of concentric knee extension, from 0.54 at 60°/s to 0.90 at 180°/s [26]. Given the very small sample size and homogeneity of environmental background, the contradictory results of the latter study should be interpreted with caution.

In a large sample of adult brother pairs, high 'upper-limit heritabilities' were noted for extension and flexion of the knee, elbow and the trunk, $h^2 = 0.63–0.87$ [27]; note, however, that estimates for brothers include an unknown shared environment effect. In another sibling study, age and sex-adjusted transmissibility of knee extension strength was 0.42 and was increased to 0.60 after adjustment for height and weight [28]. Other data indicate similar estimates of heritability for dynamic tasks, 0.60 for back extension strength in an isokinetic lifting task among 35- to 69-year-old male twins [29], and 0.46 for dynamic leg extension in postmenopausal twins which was increased after adjustment for age, height and weight [30]. Given the limited data, it is not possible to draw conclusions on potential age- or sex-associated variation in heritabilities of dynamic strength. Only one study included both men and women, but sex differences were regressed out in the analysis [28], and there are no data for adolescent boys and young adult women.

Muscular power or explosive strength as measured by jumping tasks or the Wingate test provide additional insights into the heritability of dynamic strength. In a summary of six early twin and family studies characterized by small sample sizes

and questionable zygosity determination, heritabilities for the vertical jump ranged between 0.82 and 0.93 for twin studies and 0.22 and 0.68 for family studies [31]. More recent twin studies based on the vertical and standing long jump or maximal power output in the Wingate test indicate comparable heritabilities, though analytical strategies vary. Estimated heritabilities were 0.67 for the squat jump, 0.45 for the counter-movement jump and 0.74 for maximal power developed in 5 s in a Wingate test in 16 young adult male twin pairs [32]. Among adolescent twins, an age-adjusted heritability of 0.75 was noted for the vertical jump in a sample of 54 male and female twin pairs, though possible sex differences were not considered [33], while heritabilities of 0.97 and 0.85 for peak power on a Wingate test were noted in 15 pairs of pre-adolescent and 15 pairs of adolescent female twins, respectively [26]. Two recent studies applied structural equation modeling to data for adolescents and included five zygosity groups, which permitted testing for sex differences. In a sample of 158 Japanese twin pairs 10–15 years of age, a heritability of 0.66 was obtained for the standing long jump under an AE-model with no evidence for sex differences, although age differences were not corrected [20]. In a sample of 105 male and female twin pairs of the same age, univariate cross-sectional and longitudinal models were used in genetic analyses of vertical jump [5, 34]. Sex differences were noted with the cross-sectional approach; girls showed generally higher heritabilities than boys at most measurement occasions, 0.74–0.91 compared to 0.47–0.85, respectively, with one estimate of 0.0 at 16 years [34]. With data aligned on age at PHV, sex differences were less apparent and reached significance only 3 years after PHV, 0.89 in girls and 0.61 in boys [5]. Limiting the analysis to twins at 10 years of age and including data for parents suggested some genetic dominance was included in the heritability of 0.65, which was slightly lower than a heritability of 0.72 when only twins were used in the analyses [31]. In summary, data for adolescence do not provide evidence for clear age or sex differences either, although a divergence in heritabilities is suggested towards later adolescence in Belgian twins [5]. Explosive strength data for adults are lacking. A heritability estimate of 0.32 was observed in a bivariate analysis of leg extensor power in elderly female twins; however, in a univariate analysis with an AE model which did not fit the data significantly worse than an ACE model, the heritability was 0.61 and thus more comparable to studies in adolescents [23]. Heritability appears to be somewhat higher for explosive compared with static strength, although this may be due to the fact that most evidence is derived from twin studies without inclusion of other relatives.

Motor Performance

Although jumping tasks are included in the preceding section, they are also included in studies of motor performance. Estimated heritabilities for tests of sprinting, jumping and throwing based on studies of twins and siblings of school age are variable,

ranging from 0.14 to 0.91 with no indication of sex differences [2, 35]. Most estimates are based on traditional analytical methods in contrast to more recent modeling strategies. Heritabilities for dashes vary somewhat with distance but do not support the conclusion that heritability is higher for shorter distances. An early study of parent-offspring similarities compared the performances of 24 college age men with that of their fathers when the latter were of college age 34 years earlier [36]. Correlations of 0.86 for the running long jump and 0.59 for the 100-yard dash suggest reasonably similar performances in these tasks when fathers and sons were about the same age in young adulthood. The results could be due to both inherited factors and/or the modeling of activity and sport behavior from parent to child.

Little is known of the genetic contribution to movement patterns. Results of two studies suggest a significant genotypic contribution to variation in the kinematic structure of the dash in twins of both sexes aged 11–15 years [37] and 8–14 years [38, 39]. Intrapair differences were consistently smaller in MZ compared to DZ males than in MZ compared to DZ females, suggesting that the sprinting performance of girls may be more amenable to environmental influences [37]. In contrast to the dash, intrapair differences for the kinetic structure of a throw for distance and the crawl stroke in swimming were similar in MZ and DZ twins [38, 39], suggesting an important role of environmental influences.

Balance is a skill that requires a combination of gross and fine motor control in the maintenance of equilibrium in static and dynamic tasks. Heritability estimates for various balance tests in samples of twins 8–18 years range from 0.27 to 0.86 [2, 35]. Estimates for a ladder climb and stabilometer (dynamic) are lower than those for a rail balance (static).

Flexibility measures are often indicated in performance test batteries and are joint specific. Data for biological relatives are not extensive. Estimated heritability for lower back flexibility in a sample of male twins 11–15 years was 0.69, whereas estimates for a combined sample of male and female twins 12–17 years ranged from 0.84, 0.70 and 0.91 for flexibility of the trunk, hip and shoulder, respectively. Familial correlations for lower back flexibility in biological siblings and parent-offspring pairs are low to moderate, ranging from 0.26 to 0.43 [2].

Aerobic Performance

Aerobic performance tests are both maximal and submaximal. Maximal aerobic power (maximal oxygen uptake) is measured under standardized laboratory conditions on a cycle ergometer or a treadmill and maximal or peak oxygen uptake is expressed per unit body mass. Submaximal aerobic performance is usually measured as the power output, at a heart rate of 150 or 170 beats per minute and is expressed per kg body mass (W/kg). Correlations for maximal oxygen uptake, adjusted for body mass, vary between 0.60 and 0.91 in MZ twins and between 0.04 and 0.53 in DZ twins

Table 2. Heritabilities of aerobic performance

	Twin studies	Family studies
Commonly used heritability indices		
Maximal or peak aerobic performance (adjusted for body mass)	0.40–0.94 6 studies [2]	0.38–0.66 2 studies [2]
Submaximal aerobic performance (PWC 150/kg body mass)	0.38 1 study [2]	0.50 to 0.52 3 studies [2]
Genetic determination estimated with structural equation modeling		
Maximal or peak aerobic performance		
Adjusted for body mass, lean mass or fat mass indicators	0.78 [42]	0.52 [41]
Unadjusted	0.69–0.87 [31]	–
Submaximal aerobic performance		
PWC 150/kg mass		0.28[1] [44]
PWC 110/kg mass	0.55 [40]	–
VO_2 at 60 or 80% VO_{2peak}	–	0.23–0.57[1] [45]
VO_2 at ventilatory threshold, African-American	–	0.54[1] [43]
VO_2 at ventilatory threshold, European-American	–	0.58[1] [43]

[1] Transmissibility coefficients, including both genetic and cultural transmissibility.

[2]; estimated heritabilities vary from 0.40 to 0.94 [2, 31, 40–45] (table 2) which is due to the unequal quantitative value of reported data. If studies with largest sample size and best quality control are selected, results are more concordant. Mean MZ intraclass correlations vary between 0.60 and 0.80 and mean DZ intraclass correlations vary between 0.30 and 0.50. These data are more consistent with estimates for biological siblings. More recently structural equation modeling has been applied to aerobic performance. Genetic estimates vary between 0.69 and 0.87 for adjusted and unadjusted maximal aerobic performance (table 2). Sibling correlations for submaximal aerobic performance in Canadian youth range between 0.12 and 0.45 with estimated heritabilities between 0.38 and 0.52. Estimates based on structural equation modeling vary between 0.28 and 0.55. However, spouse correlations for maximal and submaximal tests vary between 0.17 and 0.42 [2]. If the effect of this positive assortative mating is not taken into account in twin models, heritability estimates are theoretically biased downward.

Apart from studies conducted before the 1980s, most of the information on aerobic performance stems from family and twin studies conducted in Canada and Belgium. There is not enough information to verify if there are gender-specific genetic contributions and if genetic contributions vary with age. Further, adjustments for body mass, lean body mass and indicators of fatness may not be the most

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appropriate procedures to estimate the genetics of relative or normalized aerobic performance. Using a multivariate structural equation analytical protocol with muscular strength as an example, muscle cross-sectional area and isometric, eccentric and concentric strength are under the control of shared genetic and environmental factors [21]. The shared genetic factors explained significant proportions of the variance: 43% in muscle cross-sectional area and 47% in eccentric, 58% in isometric and 32% in concentric strength. The results indicate that performance and anthropometric dimensions are in part under the control of the same set of genes so that adjusting for body mass or other anthropometric dimensions could bias the estimated genetic contribution.

Responsiveness to Training

Linkage, gene association studies and more recently gene expression studies have used measured genotype approaches to explore the evidence for genotype × training interactions. Nevertheless, unmeasured genotype studies are still useful to estimate genetic variation in responses to training. Highly controlled studies with MZ and DZ twins and families have been performed using intermittent, aerobic and resistance strength training protocols.

Strength Training
Genotype × strength training interaction has been studied in only two twin designs. In 5 twin pairs who were submitted to a 10-week isokinetic knee extension/flexion strength training program, there were as many interindividual differences in response to training among members of any given pair of twins as between twin pairs [46]. There also was no clear genotype × training interaction for the responses of several metabolic enzyme levels in muscle tissue: creatine kinase, hexokinase, malate dehydrogenase (MDH) and 3-hydroxyacyl CoA dehydrogenase enzyme levels (in 4 trained MZ pairs), although significant MZ intraclass correlations were observed for the response in oxoglutarate dehydrogenase (OGDH). In a larger study of 25 MZ and 16 DZ young adult male twin pairs, both F-tests and bivariate longitudinal model fitting were used to study evidence for strength training-specific genes or genotype-dependent responses to concentric high-resistance strength training [8]. There was considerable interindividual variability in response to the 10-week resistance training protocol for the elbow flexors and the intrapair correlation for changes in 1 RM in MZ twins was 0.46. Bivariate longitudinal model fitting indicated evidence for a 'training-induced' set of genes that explained about 19–23% of the post-training variance in 1 RM, isometric and concentric strength at 120°/s arm flexion. Although there was some evidence for genotype × strength training interaction, most of the genetic variance in post-training strength measures was shared with genetic factors that also explained pre-training variability.

Intermittent and Aerobic Training

Results of studies using standardized training protocols to improve maximum oxygen uptake in MZ twins indicate a genotype × training interaction [2]. Among 10 pairs of male MZ twins submitted to a standardized laboratory-controlled training program for 20 weeks, gains in absolute VO_{2max} showed almost eight times more variance between pairs of twins than within pairs of twins [47]. A similar experiment using twice daily exercise training for 3 months at a constant energy and nutrient intake in seven pairs of young adult male MZ twins showed significant intrapair resemblance in gains in VO_{2max} [48]. In a third study, total power output during a 90-min maximal cycle ergometer test was monitored before and after 15 weeks of training in twins; similarity in response in total power output within twin pairs was significant (r = 0.83) and the F-ratio of between-pair to within-pair variances approximated 11 [49]. The evidence also suggested that changes in PFK, MDH, 3-hydroxyacyl CoA dehydrogenase and OGDH in six twin pairs were partly determined by genotype, specifically in the second half of the study (weeks 7–15). Further evidence for genotype dependency in responses to endurance training comes from the largest family-based endurance training study, 'HERITAGE Family Study'. Age- and sex-adjusted responses of VO_{2max} (ml O_2) showed a maximal heritability of 47% based on genetic model fitting analysis of 481 individuals from 98 families of European ancestry (White) including sedentary parents and their adult offspring who trained for 20 weeks [50].

In contrast, responses to 15 weeks of high-intensity, intermittent training on short-term anaerobic performance (10-second power output) of 14 male MZ twin pairs were minimally affected by genotype, while the training response to long-term anaerobic performance (90-second power output) was largely determined by genetic factors [51]. Several key enzymes showed significant intrapair correlations in response to the training protocol: creatine kinase = 0.82, hexokinase = 0.59, LDH = 0.64, MDH = 0.5, OGDH = 0.5, PFK/OGDH ratio = 0.64.

Genetic Considerations in Skill Acquisition

Genotype and genotype × environment interactions are not ordinarily considered in discussions of motor skill acquisition or in the development of expertise, although both genetic and shared environment effects are apparent in familial studies of motor performance. The potential role of genotype in motor skill acquisition (motor learning) needs attention. Several relatively dated experimental studies have considered the pattern of learning motor skills in adolescent twins [2]. Except for a stabilometer task, which places a premium on dynamic balance and coordination, tasks used in the experimental studies were largely fine motor skills that stressed manual dexterity and precision of movement. Similar rates of learning motor skills were noted in MZ and DZ twins; however, estimates of the genetic contribution varied from task to task and during the time course of learning, i.e. over practice trials or training sessions.

Detailed genetic analyses of learning curves for motor skills are limited. Parameters of the learning curves of four tasks – plate tapping with the hand, tapping with one foot, mirror tracing, and a ball toss for accuracy – in twins 9–13 years of age give variable results [52]. Two parameters, level and rate of learning, were more similar in MZ than in DZ twins, and intrapair correlations tended to be higher in male than in female MZ twins. The third parameter, the final level of skill attained, was quite variable between twin types and sexes and among the four tasks. A more recent study of adult twins reared apart, on average, about 43 ± 14 years of age, indicated significant genetic effects for rotary pursuit performance per se and rate of learning [53]. The authors suggested that practice functions to reduce the effects of environmental variance and to increase the strength of genetic effects in this motor skill.

The results, though limited, suggest that genotype may be an important determinant of the ease or difficulty with which motor skills are learned, or of improvements in performance that occur with practice. There is task specificity in heritability estimates and heritabilities differ from initial to later series of practice trials or training sessions. Factors that may condition learning of motor skills are not considered in the available studies, e.g. age, experience, current level of skill. There is a need for more detailed study of the individuality of responses to instruction and regular practice of motor tasks during childhood and adolescence, and interactions of the characteristics of the learner (which are in part genetic) and the instruction/practice environment.

Future Directions

Wide ranges of heritability estimates have been reported for several performance phenotypes. This is explained, in part, by differences in sample characteristics (family vs. twin studies, sample size, gender, age range) and data analysis (correction for covariates, classical h^2 estimates or genetic model fitting). For many performance phenotypes, conclusive data are lacking for the significance of effects of age, sex, maturation and ethnicity on heritability estimates and also for sufficiently large samples using the best available analytical techniques. To date, data are not available for performance phenotypes in populations living under marginal or compromised environmental conditions that characterize many developing countries, e.g. infectious and parasitic diseases, chronic undernutrition, and their interactions.

Although measured genotype approaches are now applied to study the role of specific sequence variants in many performance- and health-related physical fitness phenotypes [54], detailed study of the importance of genetic and environmental variation and interactions are an important prerequisite to study specific 'measured' genotype effects. The unmeasured genotype approach quantifies the importance of genetic variation in the total observed variability and may be used to 'rank' performance phenotypes by their degree of heritability to guide future efforts in gene hunting.

Multivariate models are also able to test hypotheses about the 'common action' of genes and environmental factors, which increases power to find genes with pleiotropic action. If, however, covariation between traits is due mainly to environmental factors, it may guide researchers to search for trait-specific genes rather than to focus on a search for pleiotropic genes.

MZ twin designs are very useful to study genotype × environment interaction with the unmeasured genotype approach. They are also useful in studying epigenetic variation that influences differences in levels of gene expression and might therefore help explain observed differences between MZ scores (discordance in disease status, but probably also in performance status) mediated in part by the interaction effects of physical activity and practice/training. Application of the method for testing genotype × environment interactions proposed by Purcell [9] to physical performance phenotypes may yield heritability estimates that vary with levels of physical activity or training. This approach may clarify some of the discrepancies among studies and different populations in heritability estimates for performance phenotypes.

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Genes, Athlete Status and Training – An Overview

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Abstract

Significant data confirming the influence of genes on human physical performance and elite athlete status have been accumulated in recent years. Research of gene variants that may explain differences in physical capabilities and training-induced effects between subjects is widely carried out. In this review, the findings of genetic studies investigating DNA polymorphisms and their association with elite athlete status and training responses are reported. A literature search revealed that at least 36 genetic markers (located within 20 autosomal genes, mitochondrial DNA and Y-chromosome) are linked to elite athlete status and 39 genetic markers (located within 19 genes and mitochondrial DNA) may explain, in part, an interindividual variability of physical performance characteristics in response to endurance/strength training. Although more replication studies are needed, the preliminary data suggest an opportunity to use some of these genetic markers in an individually tailored prescription of lifestyle/exercise for health and sports performance.

It has long been recognized that the interindividual variability of physical performance traits and the ability to become an elite athlete have a strong genetic basis. The genetic loci that influence these phenotypes are now being sought. As the result, several family, twin, case-control and cross-sectional studies suggested an important role of genetics along with epigenetic and environmental factors in determination of individual differences in athletic performance and training responses [1].

A quarter of a century has passed since the first use of the '*Genetics of fitness and physical performance*' term by Claude Bouchard [2] and the first publication in this field [3]; however, the era of modern genetics of physical performance began in late 1990s, when the data from the Human Genome Project acquired currency. Specifically, in 1997 Montgomery et al. [4] reported greater left ventricular (LV) hypertrophy in military recruits carrying the angiotensin I converting enzyme *(ACE)* DD genotype

compared with I allele carriers after military basic training. In 1998, Montgomery et al. [5] followed up with a report in *Nature* showing higher *ACE* I allele frequency in a group of high-altitude mountain climbers compared with controls, as well as greater exercise performance improvements in II compared with DD genotypes after exercise training. This paper provided the impetus for many other groups to test the association of this and other gene polymorphisms with various performance-related phenotypes in follow-up studies [6]. In this review, recent findings of genetic studies exploring DNA polymorphisms and their association with elite athlete status and training responses are presented.

Genes and Elite Athlete Status

A wide variety of factors determines athletic success: genetics, epigenetics, training, nutrition, motivation, advances in equipment and other environmental factors. Genetics has a great influence over components of the athletic performance such as strength, power, endurance, muscle fiber size and composition, flexibility, neuromuscular coordination, temperament and other phenotypes. Accordingly, athlete status is a heritable trait: around 66% of the variance in athlete status is explained by additive genetic factors. The remaining variance is due to nonshared environmental factors [7].

Over the decade since the *ACE* gene was first proposed to be a 'human gene for physical performance' [5], there have been numerous studies examining the effects of *ACE* and other genes on athletic status. Despite the obvious role of genetics in human athletic performance, there is little unequivocal evidence in support of a specific genetic variant with a major gene effect on a relevant performance phenotype, at least across the normal range of human trait distributions. This may be because complex traits are fundamentally polygenic (numerous genes with small effects), or because researchers failed to take into consideration the full range of environmental effects, or both [8].

Based on final effect, DNA variations generally can be classified as genetic markers associated with endurance or power athlete status [9–12], or both with endurance and power athlete status [13, 14]. Thirty-six genetic markers (located within 20 autosomal genes, mitochondrial DNA, mtDNA, and Y-chromosome) have been reported to be associated with endurance and power athlete status (table 1).

Gene Variants for Endurance Athlete Status

ACE I Allele
Circulating ACE exerts a tonic regulatory function in circulatory homeostasis, through the synthesis of vasoconstrictor angiotensin II, which also drives aldosterone

Table 1. Gene variants (genetic markers) for elite athlete status

Gene	Location	Polymorphism	Endurance- or power-related marker
Gene variants for endurance athlete status			
ACE	17q23.3	Alu I/D	I
ADRA2A	10q24-q26	6.7/6.3 kb	6.7 kb
ADRB2	5q31-q32	Arg16Gly (rs1042713 G/A)	Arg16
AMPD1	1p13	Q12X (rs17602729 C/T)	Q12
BDKRB2	14q32.1-q32.2	+9/−9	−9
EPAS1 (HIF2A)	2p21-p16	rs1867785 A/G	rs1867785 G
		rs11689011 C/T	rs11689011 T
MtDNA loci	MtDNA	haplogroups constructed from several MtDNA polymorphisms	favorable: H and L0
			unfavorable: K, J2, T and L3*
NFATC4	14q11.2	Gly160Ala (rs2229309 G/C)	Gly160
NOS3	7q36	Glu298Asp (rs1799983 G/T)	Glu298
		$(CA)_n$ repeats	164-bp
		27 bp repeats (4B/4A)	4B
PPARA	22q13.31	rs4253778 G/C	rs4253778 G
PPARD	6p21.2-p21.1	rs2016520 T/C	rs2016520 C
PPARGC1A	4p15.1	Gly482Ser (rs8192678 G/A)	Gly482
PPARGC1B	5q33.1	Ala203Pro (rs7732671 G/C)	203Pro
		Arg292Ser (rs11959820 C/A)	292Ser
PPP3R1	2p15	Promoter 5I/5D	5I
TFAM	10q21	Ser12Thr (rs1937 G/C)	12Thr
UCP2	11q13	Ala55Val (rs660339 C/T)	55Val
UCP3	11q13	rs1800849 C/T	rs1800849 T
VEGFA	6p12	rs2010963 G/C	rs2010963 C
Y-chromosomal haplogroups	Y-chromosome	haplogroups constructed from several Y-chromosome polymorphisms	favorable: E*, E3* and K*(xP)
			unfavorable: E3b1
Gene variants for power athlete status			
ACE	17q23.3	Alu I/D	D
ACTN3	11q13.1	R577X (rs1815739 C/T)	R577
HIF1A	14q21-q24	Pro582Ser (rs11549465 C/T)	582Ser
PPARA	22q13.31	rs4253778 G/C	rs4253778 C
PPARG	3p25	Pro12Ala (rs1801282 C/G)	12Ala

synthesis, and the degradation of vasodilator kinins. A polymorphism in intron 16 of the human *ACE* gene has been identified in which the presence (insertion, I allele) rather than the absence (deletion, D allele) of a 287-bp Alu-sequence insertion fragment is associated with lower serum and tissue ACE activity. An excess of the I allele has been associated with some aspects of endurance performance, being identified in 34 elite British ≥5,000 m distance runners [15] and 25 elite mountaineers [5]. In addition, an excess of the I allele is present in elite Australian (n = 64) [11], Croatian (n = 40) [16] and Russian (n = 107) [17] rowers as well as Spanish elite athletes (25 cyclists, 20 long-distance runners, 15 handball players) [18]. *ACE* I allele is also over-represented among 100 fastest Ironman triathletes [19], successful marathon runners (scoring on places from 1st to 150th) [20], 35 outstanding Russian middle-distance athletes (24 swimmers, 7 track-and-field endurance athletes, 4 cross-country skiers) [21], 33 Italian Olympic endurance athletes (10 road cyclists, 7 track-and-field runners, 16 cross-country skiers) [22], 80 Turkish endurance and power/endurance athletes (17 middle-distance running, 10 basketball, 18 handball, 35 football players) [23] and 16 long-distance (25 km) swimmers from different nationalities [24].

An excess frequency of the *ACE* I allele or II genotype in endurance-oriented athletes may be partly explained by a genotype-dependent improvement in skeletal muscle mechanical efficiency with training [25], association of the *ACE* II genotype with an increased percentage of slow-twitch type I fibers in human skeletal muscle [26], higher VO_{2max} [27], higher aerobic work efficiency [28], improved fatigue resistance [5], higher peripheral tissue oxygenation during exercise [29], greater aerobic power response to training [30], improved hypoxic ventilatory response [31], greater cardiac output and maximal power output in athletes [17, 32].

ADRA2A 6.7-kb Allele

The α-2A-adrenergic receptor (ADRA2A) plays a central role in the regulation of systemic sympathetic activity and hence cardiovascular responses such as heart rate (HR) and blood pressure (BP). The restriction enzyme *Dra*I identifies a restriction fragment length polymorphism in the 3′-untranslated region (6.7-/6.3-kb polymorphism) of the *ADRA2A* gene. Wolfarth et al. [33] have observed a significant difference in genotype distributions between elite endurance athletes (148 Caucasian male subjects) and sedentary controls (149 unrelated sedentary male subjects). A higher frequency of the 6.7-kb allele was found in athletes compared with the sedentary controls group. It was concluded that genetic variation in the *ADRA2A* gene or a locus in close proximity may play a role in being able to sustain the endurance training regimen necessary to attain a high level of maximal aerobic power [33].

ADRB2 Arg16 Allele

β$_2$ adrenergic receptor (encoded by *ADRB2*) is a member of the G protein-coupled receptor superfamily, expressed in many cell types throughout the body and play a pivotal role in the regulation of the cardiac, pulmonary, vascular, endocrine and

central nervous system. The Arg16Gly SNP (rs1042713 G/A) of the *ADRB2* gene and its association with several phenotypes has been described. Specifically, Arg16 allele is associated with lower receptor density and resting cardiac output [34]. Recently, Wolfarth et al. [35] reported that Arg16 allele was overrepresented in 313 white male elite endurance athletes compared with 297 white male sedentary controls, suggesting a positive association between the tested Arg16Gly polymorphism and endurance performance. The results of this study are in agreement with the previous work in which the association of Arg16 allele with higher peak VO_2 in heart failure patients was reported [36].

AMPD1 C34 Allele

Adenosine monophosphate deaminase 1 (AMPD1) catalyzes the deamination of adenosine monophosphate to inosine monophosphate in skeletal muscle. Deficiency of the AMPD1 is apparently a common cause of exercise-induced myopathy and probably the most common cause of metabolic myopathy in the human. In the overwhelming majority of cases, AMPD1 deficiency is due to a 34C/T transition in exon 2 (rs17602729) of the *AMPD1* gene, which creates a non-sense codon (Q12X) that prematurely terminates translation. AMPD1 deficiency individuals exhibit a low AMP deaminase activity, a faster accumulation of blood lactate during the early recovery from a 30-second sprint exercise [37] and reduced submaximal aerobic capacity (VO_2 at the ventilatory threshold, VT) [38]. In a study of Rico-Sanz et al. [39], subjects with the *AMPD1* XX genotype had diminished exercise capacity and cardiorespiratory responses to exercise in the sedentary state. Furthermore, the training response of ventilatory phenotypes during maximal exercise was more limited in XX [39]. Additionally, Fischer et al. [40] revealed a faster power decrease in the AMPD-deficient group during the 30-second Wingate cycling test. Finally, Rubio et al. [12] reported low frequency of the mutant X allele in a group of top-level Caucasian (Spanish) male endurance athletes (cyclists and runners, n = 104) compared with Spanish healthy non-athletes (n = 100).

BDKRB2 –9 Allele

Bradykinin is a potent endothelium-dependent vasodilator and acts via the bradykinin B2 receptor (encoded by *BDKRB2*). The absence (–9), rather than the presence (+9), of a 9-bp repeat sequence in exon 1 has previously been shown to be associated with increased gene transcription and higher *BDKRB2* mRNA expression. Williams et al. [41] have shown that the –9 allele of the *BDKRB2* gene is associated with higher efficiency of muscular contraction (i.e. the energy used per unit of power output during exercise or delta efficiency). In the same study of 81 elite British runners, analysis revealed a linear trend of increasing –9 allele frequency with distance run. The proportion of –9 alleles increased from 0.382 to 0.412 to 0.569 for those athletes running ≤200, 400–3,000, and ≥5,000 m, respectively [41]. The –9/–9 genotype of the *BDKRB2* gene was also overrepresented in male Caucasian triathletes (n = 443) of the

2000 and 2001 South African Ironman Triathlons compared with male controls (n = 203) [42]. Additionally, when divided into tertiles according to their finishing times, the –9/–9 genotype was only overrepresented in the fastest tertile.

EPAS1 rs1867785 G and rs11689011 T Alleles

Endothelial PAS domain protein 1 (EPAS1) is a hypoxia-inducible transcription factor and plays an important role in the catecholamine and mitochondrial homeostasis, in the control of cardiac output and erythropoietin regulation. Recently, Henderson et al. [43] have examined the impact of DNA variants within *EPAS1* (also known as *HIF2A*) gene in a physiological model such as elite endurance athletes. The frequencies of the G (rs1867785 A/G) and T (rs11689011 C/T) alleles located within the large intron 1 of the *EPAS1* gene tended to be higher in short (event duration no less than 50 s), middle (from 50 s to 10 min) and long (from ~2 to 10 h) distance Australian endurance athletes [in order of increasing frequency: 127 Ironmen triathletes, 58 cyclists, 24 Olympic distance triathletes, 172 rowers, 42 swimmers (100–800 m) and 28 middle distance runners] in comparison with 444 controls. They have also identified three *EPAS1* haplotypes to be significantly associated with elite endurance athletes classified according to the power-time model of endurance. The presence of one (haplotype G: A-T-G-G) and the absence of another (haplotype F: G-C-C-G) at the same locus was observed in athletes involved in high-intensity maximal exercise of a duration between 50 s and 10 min. In addition, athletes involved in a sustained steady-state effort (from ~2 to 10 h) demonstrated the increased presence of a third (haplotype H: A-T-G-A) [43].

MtDNA Haplogroups

Patients with mutations in mtDNA commonly present with exercise intolerance, muscle weakness and increased production of lactic acid [44]. An association has been found between several mtDNA control region polymorphisms and endurance capacity in sedentary men [45], and between morph variants of MTND5 and the level of maximum oxygen uptake [46], suggesting that certain mtDNA lineages may contribute to good aerobic performance. In a study of Finnish elite endurance athletes (n = 52), an excess of mtDNA haplogroup H and the absence of haplogroup K and subhaplogroup J2 compared with 1,060 controls and 89 sprinters was reported [44]. Haplogroup T was significantly less frequent among 95 Spanish elite endurance athletes in comparison with 250 healthy male population controls [47]. Recently, Scott et al. [48] have shown a greater proportion of L0 haplogroups and lower proportion of L3* haplogroups in 70 Kenyan elite endurance athletes compared with controls (Kenyan population, n = 85).

NFATC4 Gly160 Allele

Nuclear factor of activated T cell calcineurin-dependent 4 (NFATC4) is a transcription factor that regulates cardiac hypertrophy, muscle fiber composition, glucose and

lipid homeostasis, mitochondrial biogenesis and hippocampal neuronal signaling. *NFATC4* gene (also known as NFAT3) Gly160Ala polymorphism (rs2229309 G/C) was shown to be associated with indexes of cardiac hypertrophy [49]. Specifically, a lower mean of LV mass and wall thickness were observed in carriers of the *NFATC4* 160Ala allele. In a study of 1,580 Russian athletes, the frequency of the Gly160 allele of the *NFATC4* gene was higher in elite endurance-oriented athletes (n = 351) than in the control group [50]. Furthermore, *NFATC4* Gly allele was associated with high values of aerobic performance (VO_{2max} and AT in % of VO_{2max} values) both in male and female Russian rowers [51].

NOS3 Glu298, 164-bp and 4B Alleles

Endothelial nitric oxide synthase (NOS3) generates NO in blood vessels and is involved in regulating vascular function. The *NOS3* gene contains a number of most frequently studied polymorphisms, such as Glu298Asp (E298D or G894T or rs1799983) in exon 7, microsatellite $(CA)_n$ repeats in intron 13, and 27-bp repeats in intron 4 (4B/4A) variations. Evidence suggests that the *NOS3* 298Asp allele is associated with reduced ecNOS activity, reduced basal NO production and vascular disease in several populations. Saunders et al. [42] investigated *NOS3* Glu298Asp polymorphism (in combination with the *BDKRB2* polymorphism) in 443 male Caucasian Ironman triathletes and 203 healthy Caucasian male control subjects. There was a tendency of the *NOS3* Glu298 allele combined with a *BDKRB2* –9/–9 genotype to be overrepresented in the fastest finishing triathletes (n = 40, 28.6%) compared with the control subjects (n = 28, 17.3%; p = 0.028) [42]. In the Genathlete study, Wolfarth et al. [52] have examined the contribution of the three above-mentioned polymorphisms to discriminate 316 elite endurance athletes from 299 sedentary controls. The frequency of the most common 164-bp allele of the $(CA)_n$ repeat was significantly higher in endurance athletes in comparison with controls (p = 0.007). In a study of 168 Russian rowers [17], no difference was found between the athletes and controls for the 27-bp repeat polymorphism, although none of the highly elite rowers had the *NOS3* 4A/4A genotype which has been reported to be unfavorable for high-altitude adaptation (as well as *NOS3* Glu/Glu genotype) [53]. In addition, cross-sectional study in 27 Russian rowers revealed the association of *NOS3* 4B/4B genotype with higher aerobic capacity [17].

PPARA rs4253778 G Allele

Peroxisome proliferator-activated receptor-α (PPARα) is a transcription factor that regulates lipid, glucose and energy homeostasis. Endurance training increases the use of nonplasma fatty acids and may enhance skeletal muscle oxidative capacity by PPARα regulation of gene expression. Exercise-induced LV growth in healthy young men was strongly associated with the intron 7 G/C (rs4253778) polymorphism of the *PPARA* gene [54]. Individuals homozygous for the C allele had a 3-fold greater and heterozygotes had a 2-fold greater increase in LV mass than G allele homozygotes,

leading to the hypothesis that the hypertrophic effect of the rare intron 7 C allele is due to influences on cardiac substrate utilization. Recently, it was shown that the frequency of the intron 7 G allele was significantly higher in 491 Russian endurance-oriented athletes compared with 1,242 controls [9]. In accordance with the hypothesis, mean percentage of type I muscle fiber was higher in GG homozygotes than in CC genotype subjects (in a study of 40 physically active healthy men) [9]. Furthermore, the GG genotype was shown to be correlated with high values of oxygen pulse both in male and female Russian rowers [55].

PPARD rs2016520 C Allele

PPARδ is a transcription factor involved in regulation of genes implicated in fatty acid oxidation, cholesterol metabolism and thermogenesis. Overexpression of a constitutively active PPARδ (VP16-PPARδ) in skeletal muscles of transgenic mice preprograms an increase in oxidative muscle fibers, enhancing running endurance by nearly 100% in untrained adult mice [56]. The SNP located at the 5′-UTR region of the exon 4 (rs2016520, referred as +294 T/C or +15 C/T or c.-87T/C) variant in *PPARD* gene has been intensively studied. Skogsberg et al. [57] have shown that the rare C allele had higher transcriptional activity than the common T allele. Furthermore, the *PPARD* C allele has been reported to be significantly associated with an increased muscle glucose uptake [58], a lower BMI both in athletes and non-athletes [55, 59] and an increased proportion of slow-twitch muscle fibers [60]. In addition, a significantly higher frequency of the *PPARD* C allele was observed in long endurance (n = 308, 19%), middle endurance (n = 220, 17.5%) and short endurance (n = 81, 20.4%) Russian athletes compared with controls (n = 610, 12.1%) [10]. However, contrary to the hypothesis that *PPARD* C allele may be advantageous for the endurance performance, Hautala et al. [61] in considering only black (n = 264) subjects, have demonstrated in *PPARD* CC homozygotes a smaller endurance training-induced increase in maximal oxygen consumption and maximal power output compared with T allele carriers.

PPARGC1A Gly482 Allele

PPARγ coactivator 1α (PGC1α, encoded by *PPARGC1A*), a transcriptional coactivator of PPAR family, is involved in mitochondrial biogenesis, fatty acid oxidation, glucose utilization, thermogenesis, angiogenesis and muscle fiber-type conversion toward slow-twitch type I fibers. The minor serine-encoding allele of the common Gly482Ser SNP in *PPARGC1A* (rs8192678 G/A) is associated with reduced expression of *PPARGC1A* [62] and obesity [63]. Furthermore, the 482Ser allele has been reported to be associated with less increase in individual anaerobic threshold after 9 months of aerobic training [64], lower aerobic capacity in Russian rowers [55] and mixed group of Spanish endurance athletes, fit, and unfit Caucasian controls [65]. In addition, in two case-control studies, significantly lower frequency of 482Ser allele in Spanish (n = 104) and Russian (n = 351) elite endurance athletes has been reported [50, 65].

PPARGC1B 203Pro and 292Ser Alleles

PPARγ coactivator 1β (PGC1β, encoded by *PPARGC1B*) is expressed predominantly in heart, skeletal muscle, brown adipose tissue and brain. Recently, Arany et al. [66] have shown that transgenic expression of PGC1β causes a marked induction of mice IIX fibers, which are fast-twitch oxidative. PGC1β transgenic muscle fibers are rich in mitochondria and are highly oxidative. Consequently, these transgenic animals can run for longer and at higher work loads than wild-type animals [66]. Two missense SNPs of the *PPARGC1B* gene in relation to human physical performance have been described. The rare 203Pro allele of the Ala203Pro (rs7732671 G/C) polymorphism has been reported to be associated with reduced risk of obesity [67], enhanced insulin-stimulated glucose metabolism and protection against an age-related decline in PGC1β expression in muscle [68]. In a study of Russian elite endurance athletes (n = 351), the frequency of the 203Pro allele has been shown to be higher than in controls (n = 1,057) [50]. The second polymorphism, Arg292Ser (rs11959820 C/A) seems to be functional as well. The frequency of the minor 292Ser allele was lower among type 2 diabetes mellitus patients and higher in elite male endurance athletes from the Genathlete study (n = 316) [69] compared with controls.

PPP3R1 5I Allele

Calcineurin, a Ca^{2+}/calmodulin-dependent protein phosphatase, plays an important role in mediating the hypertrophic response. It consists of a Ca^{2+}-binding regulatory subunit, calcineurin B (protein phosphatase 3, regulatory subunit B, α-isoform; encoded by *PPP3R1*), and a catalytic subunit, calcineurin A. It is activated by sustained increase in basal Ca^{2+} concentration, a condition that occurs when the heart is under mechanical stretch or neuroendocrine stimulation. Activated calcineurin dephosphorylates the NFATs, leading to their nuclear translocation and subsequent transcriptional activation of hypertrophic genes. Tang et al. [70] have shown that the 5-bp deletion (5D) allele of 5I/5D polymorphism within the *PPP3R1* promoter region may cause excessive LV growth beyond the level appropriate for cardiac workload when exposed to severe hypertension. In a study of Russian rowers, the 5D allele of the *PPP3R1* gene has been reported to be associated with greater LV mass index both in males and females, and with lower values of maximal power output and VO_{2max} [71]. In addition, the frequency of the 5D allele was found to be significantly lower in 295 Russian endurance-oriented athletes in comparison with 1,073 controls (4.7 vs. 8.8%, p = 0.0018).

TFAM 12Thr Allele

Mitochondrial transcription factor A (encoded by *TFAM*) is a protein critical for mtDNA transcription, replication and maintenance. Aerobic exercise increases *TFAM* mRNA levels to enhance mtDNA replication [72]. The rare 12Thr allele of the *TFAM* Ser12Thr polymorphism (rs1937 G/C) was found to be overrepresented in 351 Russian elite endurance athletes compared with controls [50].

UCP2 55Val Allele

The uncoupling proteins 1, 2 and 3 (UCP1, UCP2, and UCP3) are members of the super family of anion carrier proteins located in the inner membrane of mitochondria. The UCP2 protein (encoded by *UCP2*) is involved in uncoupling oxidative phosphorylation from ATP synthesis in certain tissues and regulation of lipid metabolism and energy expenditure. A common Ala55Val polymorphism (rs660339 C/T) has been described in the *UCP2* gene and has been variably associated with altered BMI, physical activity and changes in energy expenditure [73–75]. More specifically, the Val/Val genotype has been reported to be associated with higher exercise efficiency [73], enhanced metabolic efficiency and physical activity [75] and higher VO_{2max} in 27 male Russian rowers [17]. Recently, it has been shown that the frequency of the 55Val allele is overrepresented in 351 Russian elite endurance athletes [50] compared with 1,057 controls.

UCP3 rs1800849 T Allele

The expression of *UCP3* mainly in skeletal muscle mitochondria made UCP3 an attractive target for studies toward manipulation of energy expenditure to fight disorders such as obesity and type 2 diabetes. Overexpressing human *UCP3* in mice resulted in lean, hyperphagic mice [76]. In humans, acute exercise induces up-regulation of UCP3, most likely because of elevated plasma free fatty acid levels [77, 78]. Several polymorphisms in the *UCP3* gene have been identified and related to markers of energy metabolism, aerobic capacity and obesity [17, 79, 80]. One of the early detected observations was 5′UTR -55 C/T polymorphism (rs1800849), of which the T allele was reported to be associated with increased skeletal muscle UCP3 mRNA expression [81], reduced BMI [80] and increased aerobic capacity in Russian female rowers [17]. The frequency of the *UCP3* T allele was significantly higher in 351 Russian elite endurance athletes compared with 1,057 controls [50]. In a Genathlete study, the difference in *UCP3* TT genotype frequency between 183 endurance athletes and 121 controls almost reached significance level (12 vs. 6%; p = 0.076) [82]. However, Hudson et al. [83] have found no association between the –55 C/T polymorphism within the *UCP3* gene and the ultra-endurance performance of triathletes who completed either the 2000 or 2001 South African Ironman triathlons.

VEGFA rs2010963 C Allele

Angiogenesis is a critical phenomenon in the adaptation to aerobic exercise training and mediated by a number of angiogenic factors including vascular endothelial growth factor (VEGF). VEGF mRNA is upregulated in human vastus lateralis following 30–45 min of one-legged knee extension exercise [84, 85]. The G-634C SNP (rs2010963) in the promoter region of the *VEGFA* gene has been associated with VEGF protein expression in peripheral blood mononuclear cells [86]. Two studies revealed associations of *VEGFA* gene polymorphisms with aerobic capacity in humans and endurance athlete status. Prior et al. [87] reported a promoter region haplotype (which includes rs2010963 C allele) to be associated with higher *VEGFA*

expression in human myoblasts and the maximal rate of oxygen uptake in non-athletes before and after aerobic exercise training, whilst Ahmetov et al. [88] reported a positive association between a *VEGFA* rs2010963 C allele and both elite endurance athlete status in Russians and the maximal rate of oxygen uptake in rowers.

Y-Chromosomal Haplogroups

Several positive associations have been reported between specific haplogroups of the Y chromosome and a number of phenotypes, including infertility, low sperm count, prostate cancer, BP and stature [89]. In respect to sports performance, Moran et al. [90] reported that the Y chromosome haplogroups E*, E3* and K*(xP) were significantly more frequent in the Ethiopian endurance running groups (n = 44) than in controls (95 members of the general Ethiopian population and 85 Arsi controls), whereas haplogroup E3b1 was less frequent.

Gene Variants for Power Athlete Status

ACE D Allele

The I/D polymorphism of the *ACE* gene denotes a substantial individual variation in renin-angiotensin system activity with the D allele being associated with higher ACE activity. Circulating ACE activity is significantly correlated with isometric and isokinetic quadriceps muscle strength [91]. Such effect may depend upon increased ACE-mediated activation of the growth factor angiotensin II, and increased degradation of growth-inhibitory bradykinin. Accordingly, greater training-related increases in quadriceps muscle strength [92, 93], peak elbow flexor muscle strength and biceps muscle cross-sectional area [94], and changes in LV growth [4] have been associated with the D allele. Similarly, several studies have shown the D allele to be associated with greater strength and muscle volumes at baseline [95–97] and an increased percentage of fast-twitch muscle fibers [26]. In addition, the D allele was associated with elite power athlete status [15, 21, 98].

ACTN3 R577 Allele

The α-actinins constitute the predominant protein component of the sarcomeric Z line in skeletal muscle fibers, where they form a lattice structure that anchors together actin-containing thin filaments and stabilizes the muscle contractile apparatus. Expression of the α-actinin-3 (ACTN3) is limited to fast muscle fibers responsible for generating force at high velocity. A common genetic variation in the *ACTN3* gene that results in the replacement of an arginine with a stop codon at amino acid 577 (C-to-T transition in exon 16; rs1815739; R577X) had been identified. The 577X allele contains a sequence change that completely prevents the production of functional α-actinin-3 protein. Several case-control studies reported that *ACTN3* RR genotype is overrepresented or *ACTN3* XX genotype is underrepresented in strength/sprint athletes in

comparison with controls. More specifically, Yang et al. [99] for the first time have shown that the frequency of the *ACTN3* XX genotype was reduced in Australian power athletes (6 vs. 20%) compared with controls, whereas none of the Olympians or female power athletes had an XX genotype. These findings have been supported by the independent replications in case-control studies of elite Finnish sprint athletes (frequency of the XX genotype: 0 vs. 9.2%) [44], elite Greek track and field athletes (frequency of the RR genotype: 47.94 vs. 25.97%) [100], top-level professional soccer players, participating in the Spanish Championships (frequency of the RR genotype: 48.3 vs. 28.5%) [101], elite-level strength athletes from across the United States (frequency of the XX genotype: 6.7 vs. 16.3%) [102] and Russian power-oriented athletes (frequency of the XX genotype: 6.4 vs. 14.2%) [14]. The hypothesis that *ACTN3* 577R allele may confer some advantage in power performance events was supported by several cross-sectional studies in non-athletes including mouse models of the *ACTN3* deficiency [103–110]. Additionally, Vincent et al. [106] have shown that the percentage surface and number of type IIx (fast-twitch glycolytic) fibers was greater in the RR than the XX genotype group of young healthy men. Furthermore, it is supposed that the α-actinin-3 deficiency may also negatively influence the power component of competition performance at least in Russian endurance athletes [13].

HIF1A 582Ser Allele

Glycolysis is the central source of anaerobic energy in humans, and this metabolic pathway is regulated under low-oxygen conditions by the transcription factor hypoxia-inducible factor 1α (HIF1α; encoded by *HIF1A*). HIF1α controls the expression of several genes implicated in various cellular functions including glucose metabolism (glucose transporters and glycolytic enzymes). A missense polymorphism, Pro582Ser, is present in exon 12 (C/T at bp 85; rs11549465). The rare T allele is predicted to result in a proline to serine change in the amino acid sequence of the protein. This substitution increases HIF1α protein stability and transcriptional activity, and therefore, may improve glucose metabolism. Recently, Ahmetov et al. [111] investigated a hypothesis that *HIF1A* Pro582Ser genotype distribution may differ for controls and Russian sprint/strength athletes, for which anaerobic glycolysis is one of the most important sources of energy for power performance. The frequency of *HIF1A* 582Ser allele was significantly higher in weightlifters (n = 53) than in 920 controls (17.9 vs. 8.5%; p = 0.001) and increased with their levels of achievement [sub-elite (14.7%) → elite (18.8%) → highly elite (25.0%)]. Moreover, the 582Ser allele was significantly associated with an increased proportion of fast-twitch muscle fibers in m. vastus lateralis of all-round speed skaters [111].

PPARA rs4253778 C Allele

PPARα is a ligand-activated transcription factor that regulates the expression of genes involved in fatty acid uptake and oxidation, glucose and lipid metabolism, LV growth and control of body weight. Jamshidi et al. [54] have shown that British army recruits homozygous for the rare *PPARA* C allele of the rs4253778 (intron 7 G/C)

polymorphism had a 3-fold greater increase in LV mass in response to training than G allele homozygotes. The hypothesis that intron 7 C allele is associated with the hypertrophic effect due to influences on cardiac and skeletal muscle substrate utilization was supported by the findings that *PPARA* C allele is overrepresented in 180 Russian power-oriented athletes (27.2 vs. 16.4%, p = 0.0001, in comparison with 1,242 controls) and associated with an increased proportion of fast-twitch muscle fibers in m. vastus lateralis of 40 male controls [9].

PPARG 12Ala Allele

PPARγ (encoded by *PPARG*) plays a critical physiological role as a central transcriptional regulator of adipogenic and lipogenic programs, insulin sensitivity and glucose homeostasis. The 12Ala variant of the *PPARG* gene Pro12Ala polymorphism (rs1801282 C/G) is associated with decreased receptor activity [112], improved insulin sensitivity [112] and increased BMI in humans [55, 113]. The carriers of the 12Ala allele show better glycaemic response to exercise training [114], higher rates of skeletal muscle glucose uptake [115] and greater cross-sectional area of muscle fibers [116]. In a study of Russian power-oriented athletes (n = 260), a higher frequency (23.8 vs. 15.1%, p < 0.0001) of the *PPARG* 12Ala allele compared with 1,073 controls has been reported [116].

Combined Impact of Gene Variants on Elite Athlete Status

Human physical performance phenotypes are classical quantitative traits influenced by many gene variants and environmental factors. It is very important to note that each DNA locus can explain a very small proportion of the phenotypic variance (~0.1 to ~1%). Therefore, very large sample sizes are needed to detect associations and various combinatorial approaches should be used. Until now, few studies have sought to define or quantify the impact of multiple genotype combinations that influence human physical performance [17, 41, 42, 50, 117–119].

Williams et al. [41] have shown the evidence for interaction between *BDRRB2* –9/+9 and *ACE* I/D genotypes in 115 British subjects, with individuals who were carriers of *ACE* II + *BDRRB2* –9/–9 genotype combination having the highest efficiency of muscular contraction before training. Furthermore, the *ACE*(I)/*BDRRB2*(–9) ('high kinin receptor activity') haplotype was significantly associated with endurance event among elite British athletes (p = 0.003) [41]. Saunders et al. [42] found that the *NOS3* Glu298 allele combined with a *BDKRB2* –9/–9 genotype was overrepresented in the fastest finishing Ironman triathletes (28.6%) compared with the control subjects (17.3%; p = 0.028). Gómez-Gallego et al. [117] have shown that professional road cyclists with the most strength/power-oriented genotype combination (*ACE* DD + *ACTN3* RR/RX), had higher respiratory compensation threshold values than those with the intermediate combinations (II + RX/RR, p = 0.036; DD + XX, p = 0.0004) but similar to those with the II + XX genotype combination. In a study of 173 Russian

rowers, the prevalent combination of *ACE* I/D, *ACTN3* R577X and *PPARA* intron 7 G/C genotypes in all groups was ID-RX-GG, and its frequency in elite rowers was different compared with controls (28.6 vs. 17.3%) [119]. Further, the total frequency of the *ACE* I, *ACTN3* R577, *UCP2* 55Val and *UCP3* rs1800849 T alleles in highly elite Russian rowers was 57.1% [p = 0.027 in comparison with controls (41.2%)] [17]. An increasing linear trend of the favorable for rower performance total allele frequency with increasing level of achievement has also been reported [41.9% (nonelite) → 43.0% (sub-elite) → 45.8% (elite) → 57.1% (highly elite)] [17]. In a study of 8 gene polymorphisms in 1,580 Russian athletes and 1,057 controls, it was shown that 66.7% of highly elite endurance-oriented athletes were carriers of 8–12 endurance-related alleles (*NFATC4* Gly160, *PPARGC1A* Gly482, *PPARGC1B* 203Pro, *PPP3R1* 5I, *TFAM* 12Thr, *VEGF* rs2010963 C, *UCP2* 55Val, *UCP3* rs1800849 T), while there were only 18.1% of such persons in the control group (p < 0.0001) [50].

Genes and Response to Training

Regular exercise brings about various effects on the human body including increases in endurance capacity and muscle strength/volume, and improvements in arterial stiffness. The effects from exercise differ greatly among individuals, depending on lifestyle factors and genetic backgrounds. An understanding of genetic backgrounds will help to clarify criteria of daily physical activities and appropriate exercise for individuals including athletes, making it possible to apply individualized preventive medicine and medical care. Moreover, health benefit will result from enhanced health, physical functioning and lifestyle improvement [120].

The process of exercise-induced adaptation in skeletal muscle involves a multitude of signaling mechanism initiating replication of specific DNA genetic sequences, enabling subsequent translation of the genetic message and ultimately generating a series of amino acids that form new proteins. The functional consequences of these adaptations are determined by training volume, intensity and frequency, and the half-life of the proteins [121]. It should be noted that at the molecular and cellular levels individuals with the same genotype respond more similarly to training than those with different genotypes, indicating that genes play an important role in determination of individual differences in response to training. The search for genetic markers of trainability status will likely be more productive than the investigation of molecular markers of the performance phenotype in the untrained state [122].

Candidate Genes for Response to Endurance Training

Prolonged endurance training elicits a variety of metabolic and morphological changes, including mitochondrial biogenesis, fast-to-slow fiber-type transformation

and substrate metabolism. Endurance adaptation results in increased muscle glycogen stores and glycogen sparing at submaximal lactate kinetics and morphological alterations, including greater type I fiber proportions per muscle area, and increased capillary and mitochondrial density. Repeated bouts of endurance exercise result in altered expression of a multiplicity of gene products, resulting in an altered muscle phenotype with improved resistance to fatigue [123]. In addition, HR and BP recovery acceleration after exercise are also important cardiovascular adaptations to endurance training. For instance, postexercise HR recovery improves after endurance training in sedentary healthy subjects and patients with cardiovascular diseases; however, a substantial heritable component may also be involved in the regulation of HR behavior in response to training, and this may partly explain the large interindividual variation in HR recovery. Several studies reported the associations of gene polymorphisms with postexercise HR and BP recovery [reviewed in 1].

Research on aerobic endurance clearly shows that some individuals respond more to training than others [124]. In the same study, the maximal heritability estimate of the VO_{2max} response to training adjusted for age and sex was reported to be 47%. Genetically gifted athletes have a much greater response to training. Accordingly, evidence that many of the world's best endurance runners originate from distinct regions of Ethiopia and Kenya, rather than being evenly distributed throughout their respective countries appears to further sustain the idea that the success of East African runners is genetically mediated [125]. Studies have shown that African distance runners have reduced lactic acid accumulation in muscles, increased resistance to fatigue, and increased oxidative enzyme activity, which equates with high levels of aerobic energy production. It has been proposed that these geographical disparities in athlete production may reflect a genetic similarity among those populating these regions for an athletic genotype and phenotype [126]. There are at least 29 genetic markers (located within 12 autosomal genes and MtDNA) that have been shown to be associated with endurance phenotypes in response to training (table 2).

ACE is the most extensively studied gene in genetics of fitness and physical performance. The wide distribution and multifunctional properties of the angiotensin-converting enzyme in skeletal muscles and cardiovascular system suggest that ACE could be involved in various physiological conditions. Montgomery et al. [5] reported greater exercise performance improvements (duration of repetitive elbow flexion performance with a 15-kg barbell) in British army recruits with *ACE* II compared with DD genotypes after exercise training. Improvement was 11-fold greater for those of II than for those of DD genotype. In the following study of army recruits, Williams et al. [25] have shown that the presence of the *ACE* I allele may confer an enhanced mechanical efficiency in trained muscle (by the means of 11-week program of primarily aerobic physical training). Two studies reported the association of *ACE* genotypes with aerobic performance in a disease state. More specifically, Defoor et al. [30] have shown that aerobic power response to training was greater in patients with *ACE* II than in those with the D allele in coronary artery disease patients. In addition, the *ACE* I allele was associated with an

Table 2. Gene variants (genetic markers) for endurance/strength/anaerobic power trainability status

Gene	Location	Polymorphism	Allele (genotype/haplotype) associated with an increase in endurance/power phenotype
Gene variants for endurance trainability status			
ACE	17q23.3	Alu I/D	I
		T-3892C	T
AMPD1	1p13	Q12X (rs17602729 C/T)	Q12
APOE	19q13.2	Arg158Cys	158Cys (ApoE*2)
		Cys112Arg	112Arg (ApoE*4)
ATP1A2	1q21–q23	8.0/3.3 kb (α_2 exon 1)	8.0 kb
		10.5/4.3 kb (α_2 exon 21–22)	10.5 kb
CKM	19q13.2–q13.3	*Nco*I A/G	favorable: AG
			unfavorable: GG
GABPB1 (NRF2)	15q21.2	rs12594956 A/C	rs12594956 A
		rs8031031 C/T	rs8031031 T
		rs7181866 A/G	rs7181866 G
HBB	11p15.5	–551C/T	–551C
		+16C/G (rs10768683)	+16C
HIF1A	14q21-q24	Pro582Ser (rs11549465 C/T)	Pro582
MtDNA loci	MtDNA	variants at positions 16133, 12406, 13365, 13470, 15925, 16223, 16362	favorable: non-Cam at 16133, 16223, 16362
			unfavorable: non-Cam at 12406, 13365, 13470, 15925
NRF1	7q32	rs2402970 C/T	rs2402970 C
		rs6949152 A/G	rs6949152 A
PPARD	6p21.2-p21.1	rs2016520 T/C	rs2016520 T
		rs2267668 A/G	rs2267668 A
PPARGC1A	4p15.1	Gly482Ser (rs8192678 G/A)	Gly482
VEGFA	6p12	(C–2578A)/(G–1154A) / (G–634C) combinations	AAG and CGC haplotypes
Gene variants for strength/anaerobic power trainability status			
ACE	17q23.3	Alu I/D	D (dynamic strength), I (isometric strength)

Table 2. Continued

Gene	Location	Polymorphism	Allele (genotype/haplotype) associated with an increase in endurance/power phenotype
ACTN3	11q13.1	R577X (rs1815739 C/T)	R577
IGF1	12q22-q23	(CA)$_n$ (>192/192/<192)	192
IL15	4q31	rs1057972 A/T	rs1057972 T
IL15RA	10p15-p14	rs2296135 A/C	rs2296135 C
PPP3R1	2p15	promoter 5I/5D	5I/5I (in combination with *IGF1* 192 allele)
RETN	19p13.2	−420 C/G	−420 C
		398 C/T	398 C
		540 G/A	540 G
TNF	6p21.3	−308 G/A	−308 A

enhanced exercise capacity response to physical training in chronic obstructive pulmonary disease patients [127]. Cam et al. [128] in a study of 55 female nonelite Caucasian Turkish athletes, investigated the association between *ACE* genotype and short- and medium-duration aerobic endurance performance (HR reserve parameters and 30 min running speed) improvements in response to training regimen. It has been shown that the *ACE* II genotype was associated with better improvements in medium duration aerobic endurance performance whilst *ACE* DD genotype was supposed to be more advantageous in performance enhancement in shorter duration and higher intensity endurance activities. However, in contrast to the above-mentioned studies, Rankinen et al. [129] reported a greater VO_{2max} increase with training in the DD homozygotes in comparison with II homozygotes. Recently, Bae et al. [130] have shown the association of the TT genotype for the *ACE* T-3892C polymorphism with a greater VO_{2max} increase in response to 12-week endurance training in Korean women.

AMPD is an important regulator of muscle energy metabolism: by converting AMP into inosine monophosphate with liberation of ammonia, this enzyme displaces the equilibrium of the myokinase reaction towards ATP production. Rico-Sanz et al. [39] reported that subjects with the unfavorable XX genotype (nonsense Q12X (C34T) mutation) at the *AMPD1* gene had diminished exercise capacity and cardiorespiratory responses to exercise in the sedentary state. Furthermore, the 20 week endurance training response of ventilatory phenotypes during maximal exercise was more limited in such subjects.

Apolipoprotein E (ApoE) is essential for the normal catabolism of triglyceride-rich lipoprotein constituents and plays less defined roles in skeletal muscle and nervous tissue. The *APOE* gene is polymorphic with three major alleles, *APOE**2 (158Cys), *APOE**3 (Arg158 or Cys112), *APOE**4 (112Arg), which translate into three iso-forms of the protein: normal – ApoE-ε3; dysfunctional – ApoE-ε2 and ApoE-ε4. Interestingly, Thompson et al. [131] have shown that after 6 months of exercise training VO_{2max} increased only by 5% in the *APOE* 3/3 subjects versus 13% in the 2/3 and 3/4 subjects (p < 0.01).

The Na^+-K^+-ATPase α_2 (encoded by *ATP1A2* gene) plays an important role in the maintenance of electrolyte balance in the working muscle and thus may contribute to endurance performance. Rankinen et al. [132] investigated the association between 8.0/3.3 kb (α_2 exon 1) and 10.5/4.3 kb (α_2 exon 21–22) polymorphisms of the *ATP1A2* gene and the response of VO_{2max} and maximal power output to 20 week of endurance training in 472 sedentary Caucasian subjects. Sibling-pair linkage analysis revealed associations of 8.0-kb and 10.5-kb alleles with greater increase of VO_{2max} in response to training.

The muscle-specific creatine kinase (CKM) enzyme is bound specifically to the M line of the myofibril subfragment, one of the heavy meromyosin in the vicinity of the myosin ATPase and to the outer membrane and vesicles of the sarcoplasmic reticulum, which might change the Ca^{2+} uptake and power of muscle [133]. Four studies aimed to investigate the association between *CKM* gene *Nco*I A/G polymorphism in the 3′ untranslated region and VO_{2max} responses to endurance training. Rivera et al. [134] reported a significantly lower ΔVO_{2max} to endurance training in both 160 Caucasian parents and 80 unrelated adult offspring with the rare GG genotype. In the following study of 277 full sib-pairs from 98 Caucasian nuclear families, the same group supported the hypothesis that the *CKM Nco*I A/G polymorphism may contribute to individual differences in the VO_{2max} response to endurance training [135]. However, Defoor et al. [136] have not observed the association between *Nco*I genotypes and aerobic power in 927 Caucasian coronary artery disease patients. On the other hand, Zhou et al. [133] reported greater improvement of running economy (RE) indexes (indicators of submaximal aerobic capacity) after 18-week 5,000 m running program in *CKM* AG heterozygotes than those with AA and GG genotypes.

The GA binding protein transcription factor, β-subunit 1 (GABPB1, according to the new nomenclature, August 2008, also known as NRF-2) protein is involved in activation of cytochrome oxidase expression and nuclear control of mitochondrial function. He et al. [137] have examined the association between the rs12594956 A/C, rs8031031 C/T and rs7181866 A/G polymorphisms in the *GABPB1* gene and endurance capacity at baseline and after an 18-week endurance training program in young Chinese men. The association between rs12594956 A, rs8031031 T, rs7181866 G alleles and higher training response in VO_{2max} at RE was reported. Moreover, those men carrying the ATG haplotype had 57.5% higher training response in VO_{2max} at RE than noncarriers.

Hemoglobin is the iron-containing oxygen transport metalloprotein in the red blood cells and has a crucial role in the determination of cardiorespiratory fitness. The α *(HBA)* and β *(HBB)* loci determine the structure of the two types of polypeptide chains in adult hemoglobin. He et al. [138] reported greater improvement of RE indexes after 18-week 5,000 m running program in *HBB* -551CC (−551C/T polymorphism) and +16CC (rs10768683 +16C/G polymorphism) subjects (military police recruits of Chinese Han origin) than those with other genotypes.

HIF1 is a transcription factor that acts to regulate the transcription of numerous genes involved in the processes of erythropoiesis, angiogenesis, and metabolism. HIF1 is composed of two subunits, one of which (HIF1α; encoded by *HIF1A*) is regulated by hypoxia. Prior et al. [139] have shown that *HIF1A* Pro/Pro homozygotes of the Pro582Ser polymorphism showed preservation of the ability to increase VO_{2max} through aerobic exercise training at each age (55, 60 and 65 years) level evaluated. Contrary to this, subjects carrying the Ser allele were able to increase VO_{2max} to a similar extent as Pro/Pro homozygotes at 55 years of age, but showed significantly less increase in VO_{2max} to aerobic exercise training than Pro/Pro homozygotes at 60 and 65 years of age.

Aerobic ATP generation by oxidative phosphorylation in the mitochondrial respiratory chain is a prerequisite for prolonged muscle exercise. Out of 480 subunits of the respiratory chain enzymes, 13 are encoded by mtDNA that is a 16,568-bp maternally inherited genome [44]. Dionne et al. [46] in a 12- to 20-week endurance training program study of 46 sedentary males, have shown that subjects with non-Cambridge (non-Cam) sequence variants at nucleotide position of 12406, 13365, 13470 or 15925 had lower trainability, whereas those with the non-Cam at 16133 had greater trainability. In another study of 55 sedentary males, Murakami et al. [45] reported a greater increase in VO_{2max} in response to 8-week endurance training program in subjects with non-Cam variants at 16223 and 16362.

The biogenesis of mitochondria requires the expression of a large number of genes, most of which reside in the nuclear genome. Nuclear respiratory factor 1 (encoded by *NRF1*, location: 7q32) acts on nuclear genes encoding respiratory subunits and components of the mitochondrial transcription and replication machinery [140]. Data from the HERITAGE Family Study showed suggestive evidence of linkage between baseline levels of maximum oxygen uptake (VO_{2max}) in healthy sedentary African-Americans and 7q32 [141]. He et al. [142] assessed the effect of 18 weeks of endurance training on the possible association between *NRF1* genotypes (rs2402970 C/T and rs6949152 A/G) and aerobic phenotypes (VO_{2max}, VT and metabolic cost of running at submaximum intensities, RE) in base-like state and in response to endurance training. They have found a greater trainability expressed as VT or RE in subjects with rs2402970 CC and rs6949152 AA genotypes.

PPARδ is a key regulator of fatty acid oxidation and energy uncoupling. Hautala et al. [61] in a study of black subjects reported a smaller endurance training-induced increase in aerobic capacity (VO_{2max} and maximal power output) in *PPARD* rs2016520

CC homozygotes compared with T allele carriers. Additionally, Stefan et al. [64] have shown the association of the *PPARD* rs2267668 A allele (A/G polymorphism) and a greater increase in individual anaerobic threshold after 9 months of aerobic training in sedentary subjects.

PPARγ coactivator 1α (encoded by *PPARGC1A*) has been established as an important regulator of mitochondrial content in skeletal muscle due to its apparent coactivation of multiple mitochondrial transcription factors. The rare 482Ser allele of the Gly482Ser SNP in *PPARGC1A* (rs8192678 G/A) has been reported to be associated with less increase in individual anaerobic threshold after 9 months of aerobic training in sedentary subjects [64].

VEGF is important for the maintenance of the skeletal muscle capillary supply, as well as the expansion of the capillary supply in response to exercise training. Prior et al. [87] tested the hypothesis that *VEGFA* promoter polymorphisms (C-2578A, G-1154A, G-634C) are related to *VEGFA* gene expression in human myoblasts and VO_{2max} before and after aerobic exercise training. Accordingly, they reported a higher *VEGFA* expression in human myoblasts with AAG and CGC haplotypes and a greater VO_{2max} in subjects with AAG and CGC haplotypes before and after aerobic exercise training.

Candidate Genes for Response to Resistance/Anaerobic Power Training

Adaptation to resistance training includes increased protein synthesis via regulatory changes in transcriptional and translational mechanisms, and in the production of muscle cells that are added to existing myofibers or combine and form new contractile filaments, each providing additional contractile machinery with which to generate force [143]. Significant intersubject variation is observed in the trainability of strength and anaerobic power characteristics. Evidence for a genotype-environment interaction was found for the increase in one repetition maximum (1RM), static strength, and concentric flexion at 120°/s after 10 weeks of high resistance strength training of arm flexors in male twins [144]. Studies also suggest that isometric and concentric muscle strength are under moderate to high genetic control with heritabilities ranging between 44 and 78% for isometric and 31 and 61% for concentric strength [145]. Several studies reported the association between individual differences in strength/anaerobic power phenotypes in response to resistance/anaerobic power training and gene variations (at least 10 genetic markers within 8 genes), though less frequently than the study on endurance performance (table 2).

Folland et al. [92] examined the effect of *ACE* genotype upon changes in strength of quadriceps muscles in response to 9 weeks of specific strength training of male subjects. They found a greater strength gains in subjects with the D allele. Giaccaglia et al. [93] studied muscle strength before and after an 18-month light weight lifting training and walking in older men and women in relation to *ACE* genotype. Following exercise

training, individuals with the DD genotype showed greater gains in knee extensor strength compared with II individuals. Cam et al. [128] investigated the impact of the *ACE* I/D variant in a nonelite female cohort on the trainability of running speeds. It was concluded that the *ACE* DD genotype seems to be more advantageous in performance enhancement in shorter duration and higher intensity endurance activities. Pescatello et al. [94] studied the associations among *ACE* I/D polymorphism and the response to a 12-week unilateral, upper-arm resistance training program in the trained and untrained arms of 631 men and women. They reported greater improvements in maximal voluntary contraction (MVC; isometric strength) in the trained and untrained arms after training in carriers of the I allele. On the other hand, 1RM (dynamic strength) and muscle cross-sectional area increases were greater for *ACE* D allele carriers in response to untrained arm training.

Two studies examined the *ACTN3* R577X genotype in relation to a variety of strength-related measures in response to training. Clarkson et al. [103] studied associations between *ACTN3* genotype and muscle size and elbow flexor isometric and dynamic strength in a large group of men (n = 247) and women (n = 355) before and after a 12-week elbow flexor/extensor resistance training program. Women homozygous for the *ACTN3* X allele had lower baseline MVC compared with heterozygotes. Furthermore, women with the XX genotype demonstrated greater absolute and relative 1RM gains compared with the carriers of the RR genotype after resistance training. Delmonico et al. [104] investigated knee extensor concentric peak power (PP) before and after a 10-week unilateral knee extensor strength training intervention in older men and women. In men, absolute PP change with strength training in the RR homozygotes approached a significantly higher value than in the XX group. In women, relative PP change with strength training in the RR group was higher than in the XX group.

Insulin-like growth factor-I (encoded by *IGF1*) is a potent mitogen and anabolic agent important in the growth of various body tissues, including skeletal muscle. In a 10-week unilateral strength training study with 67 older Caucasian men and women, Kostek et al. [146] reported that carriers of the 192 allele of the *IGF1* CA repeat promoter polymorphism showed greater quadriceps-muscle strength gains compared with noncarriers. In a second study with 128 white and black men and women, Hand et al. [147] investigated the effect of *IGF1* CA repeat and *PPP3R1* I/D polymorphisms on the changes in strength and muscle volume in response to a 10-week single-leg knee extension strength training program. There was a significant combined gene effect, including both *IGF1* CA repeat main effect and *IGF1* CA repeat x *PPP3R1* I/D gene x gene interaction effect, on change in strength with training. Specifically, individuals homozygous for *PPP3R1* II who were also heterozygous for 192 allele for *IGF1* had significantly greater increases in strength and muscle volume with training than those homozygous for *PPP3R1* II, who were also noncarriers of the 192 allele for *IGF1*.

Interleukin-15 is one of the most abundant cytokines in skeletal muscle with anabolic properties. Pistilli et al. [148] examined associations between interleukin-15

(IL15) and IL-15 α-receptor (IL15RA) gene polymorphisms with muscle and strength phenotypes at baseline and in response to a 12-week unilateral resistance training of the nondominant arm of Caucasians. They found gender-specific associations of IL15 rs1057972 A/T and IL15RA rs2296135 A/C SNPs with the pre- to posttraining absolute difference in dynamic and isometric strength, respectively. The T allele of the 3′UTR of the rs1057972 IL15 SNP was associated with greater improvements in posttraining 1RM in males, whilst the C allele in the 3′UTR of the rs2296135 IL15RA SNP was associated with greater improvements in posttraining isometric strength in females. The same group investigated six SNPs in the resistin (RETN; involved in the regulation of energy balance) gene in relation to muscle phenotypes in 482 subjects who performed 12-week unilateral resistance training of the nondominant arm [149]. Three RETN SNPs (–420 C/G, 398 C/T, 540 G/A) were associated with strength phenotypes among subjects when stratified by BMI. Specifically, men with a BMI ≥25 that were homozygous for the mutant allele – 420 (GG) had lower increases in 1 RM strength and MVC when compared with those that were homozygous CC. In addition, overweight Caucasian men that were homozygous for 540 AA had lower increases in 1RM strength when compared with those that were homozygous G/G. Finally, after training, heterozygous (CT) for the 398 C/T SNP Caucasian women showed less strength improvement when compared with the homozygous CC women.

In a study by Nicklas et al. [150], the association of tumor necrosis factor-α (suppresses protein synthesis and induces catabolism of skeletal muscle) gene (TNF) –308 G/A polymorphism with stair-climb time in response to walking and weightlifting training was reported. There was a significant interaction between TNF genotype and exercise on 6-month change in stair-climb time, such that individuals with –308A allele showed greater improvement in response to exercise.

Conclusions

Physical performance is a complex phenotype influenced by multiple environmental and genetic factors, and variation in human physical performance and athletic ability has long been recognized as having a strong heritable component. The question is no longer whether or not there is a genetic component to athletic potential and endurance/strength trainability, but exactly which genes are involved and by which mechanisms and pathways they exert their effect. Our current progress towards answering these questions still represents only the first steps towards a complete understanding of the genetic factors that influence human physical performance. The next decade will be an exciting period for genetics of fitness and physical performance, as we apply the new technologies of genomics (like multistage genome-wide association studies) and bioinformatics to further dissect and analyze the genetic effects on human physical ability.

The current review provides evidence that at least 36 genetic markers (located within 20 autosomal genes, mtDNA and Y-chromosome) are linked to the elite athlete

status and 39 genetic markers (located within 19 autosomal genes and mtDNA) may explain, in part, an interindividual variability of endurance/strength/anaerobic power phenotypes in response to different types of training. However, it should be emphasized that most of the case-control and association studies have not yet been replicated in independent samples. Further, each contributing gene can explain only a small portion of the observed interindividual differences in training-induced effects, and there is still no evidence that any of the identified variants have any substantial predictive value for prospectively identifying potential elite athletes. The issues with respect to appropriate study designs, sample size, population stratification and quality of the genotype/phenotype measurement are also of great importance.

The impact of genetics in sports and exercise appears to have multiple influences. Its positive effect on exercise performance must be combined with effective training programs and favorable lifestyle habits for success in sports and health benefits. Accordingly, one of the applications of sports genetics could be the development of predictive genetic performance tests. Genetic and nongenetic performance tests are similar in some aspects; however, there are two important differences. First, the same genetic markers may predict not only performance potential but also the risk of disease. Second, genetic tests can be carried out as soon as genomic DNA is obtained and at a very early age before physical qualities are developed. Recently, an Australian rugby league team claimed it has gained a competitive edge over its rivals by using genetic tests to tailor its players' training programs. The Sea Eagles, a professional rugby team in Manly, a seaside suburb of Sydney, has DNA tested 18 of its 24 players for 11 exercise-related genes [151]. Each player's training program was being redesigned according to whether his individual genetic profile indicates, for example, that his main strength is endurance or speed. As such, the application of genetic testing in sports could provide new opportunities for sports clubs to understand athletes' susceptibility for certain pathological states (injuries, cardiomyopathies, sudden death etc.), map genetic suitability for specific team positions and roles, and to gain insights into athletes' development in various sports or physical activities.

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Angiotensin-Converting Enzyme, Renin-Angiotensin System and Human Performance

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Abstract

The angiotensin-converting enzyme (ACE) I/D polymorphism is strongly associated with circulating
ACE levels in European populations. Initial studies over 10 years ago suggested an association
between the I-allele (associated with low circulating ACE activity) and elite endurance performance.
Subsequent studies also then suggested an association of the D-allele (high circulating ACE) with
power-oriented performance. Not all studies are concordant however. The published literature is
beset with the problems attendant in interpreting population-association studies including case
definition, adequate cohort size, selection bias and appropriate control groups. Notwithstanding
this, a plethora of data has been published. This has been followed by various studies trying to eluci-
date a physiological mechanism for such an association. This chapter will review the available data
regarding the ACE I/D polymorphism and human performance both at sea level and at high altitude.
It will also evaluate any data pertaining to postulated mechanisms.

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Although several candidate genes have been studied in association with human per-
formance, none has created as much debate, or consumed as many publication pages,
as an angiotensin-converting enzyme (ACE) insertion-deletion polymorphism in
intron 16. The particular polymorphism is characterised by the presence (insertion,
I-allele) or absence (deletion, D-allele) of a 287-bp *Alu* repeat sequence [1]. We all
carry two versions of the ACE-allele and in 1,906 British males free from cardio-
vascular disease the presence of II, ID and DD genotypes has been reported as 0.24,
0.50 and 0.26, respectively (I-allele frequency = 0.49) [2]. In Caucasian Europeans,
the ACE genotype frequencies for II, ID and DD genotypes are generally found in
the ratio of 1:2:1 [3]. However, the ratio of genotypes carried does vary depending
on the population studied. There is a trend toward a higher frequency of the D-allele
in Nigerians [3], whereas in Samoans and Yanomami Indians there is a much higher
frequency of the I-allele [3], and in Pima Indians the I-allele frequency is as high as

0.71 [4]. Within the UK, there are also significant differences with a higher frequency of the D-allele in Afro-Caribbean people [5] and a much higher frequency of the II genotype in UK-domiciled South Asians than Caucasians [6].

Although the ACE I/D polymorphism occurs in an intron, it is an exceptionally strong and consistent marker for ACE activity in many different Caucasian populations [7–13]. ACE is consistently highest in the DD subjects, intermediate in the ID and lowest in the II subjects. Although the polymorphism accounted for 18–20% of the variation in ACE in Caucasians of the Ectim study [7], 30–40% in Scandinavian Caucasians [10] and 47% in the Caucasians studied by Rigat [1], there are marked racial differences. In Pima Indians, the ACE I/D polymorphism accounts for only 6.5% of the variation in serum ACE activity [4], 13% in Kenyans [14] and there is no relationship apparent at all in African-Americans [15] or black South Africans [16]. The variance in the proportion of ACE activity associated with the ACE I/D polymorphism in different populations may suggest that there are racial differences in the transcriptional regulation of ACE and that the association with ACE activity is mediated through linkage disequilibrium with an as yet unknown neighbouring functional quantitative trait locus. Nonetheless, in European Caucasian populations it remains a strong and consistent marker for variation in ACE activity. There are also intrinsic differences in the renin-angiotensin system (RAS) between racial groups which make direct comparison between studies and extrapolation from studies of Caucasian subjects difficult. A lower plasma rennin activity is typically found in Black people compared with Caucasians [17, 18], and a lower plasma aldosterone level is found in Black children [19].

It is this racial difference in the RAS coupled with differences in gene frequency and the association between the ACE I/D polymorphism and ACE levels that may be one of the important keys to interpreting the different results from various studies.

The ACE I/D Polymorphism and Human Performance at Sea Level: Cross-Sectional Studies

Just over 10 years ago, the I-allele was associated with endurance performance, initially being found with excess frequency in elite distance runners [20] and rowers [21]. All of the 64 Caucasian (43 male, 21 female) Australian national rowers attending their pre-Olympics selection trial were examined for their frequency of the ACE I/D polymorphism [21]. They were a nationally representative group of elite rowers with selection bias reduced in view of their national identification programme screening for talented individuals (41 of the cohort won more rowing medals in the 1996 Olympics than any other nation). In comparison with a well-matched control group there was a clear excess of the I-allele and the II genotype reported.

The following year, a study that had targeted potential Olympic athletes was published [20]. 495 potential Olympic competitors formed the cohort. Of this total

91 were runners over distances from 100 m to 100 km, and in comparison with a large control group there was a significant excess of both the I-allele and II genotype reported due to a skew toward the I-allele in the ≥5,000 m group. Examination of the relative distribution of alleles by duration of event (≥200 m mainly anaerobic, 400–3,000 m mixed aerobic and anaerobic, and ≥5,000 m predominantly aerobic or endurance) was informative. There was a significant linear trend of increasing I-allele frequency with distance run with the proportion of I-alleles increasing from 0.35 to 0.53 and 0.62 amongst those running ≤200, 400–3,000 and ≥5,000 m, respectively. There was also a skew in favour of the D-allele amongst the sprinters (D-allele frequency 0.62 in those running ≤200 m). This was the first study to suggest that the ACE I/D polymorphism was not simply a ubiquitous 'gene marker of performance' but that the I-allele may confer a benefit in more endurance-related events and the D-allele in more power-oriented events.

At around the same time, other workers found, and published, no such association between the ACE I/D polymorphism and elite human performance [22–24]. Taylor et al. [23] examined 120 Australian national athletes from sports deemed to demand a high level of aerobic fitness (26 hockey players, 25 cyclists, 21 skiers, 15 track and field athletes, 13 swimmers, 7 rowers, 5 gymnasts and 8 'other'). They found no difference in ACE genotype and allele frequency compared with controls. Similarly, the cohort examined by Karjalainen et al. [22] of 80 elite endurance athletes from Finnish national teams included the various disciplines of long distance running, orienteering, cross-country skiing and the triathlon. Again, they found no difference in the ACE I/D polymorphism between the athletes and controls. One of the largest (n = 192) studies to date of national and international athletes (cross-country skiing, biathlon, Nordic combined, long distance running, middle distance running and road cycling) was also negative [24].

However, a key consideration of genetic association studies is case definition. The common denominator among these early negative studies was the inclusion of athletes from mixed sporting disciplines, thereby producing phenotypic heterogeneity and obscuring any possible genetic association.

To clarify this point, a study specifically addressed this issue [25]. A mixed cohort of 217 Russian athletes of regional or national standard were recruited from the following sports: swimming (n = 66), track and field athletics (n = 81), cross-country skiing (n = 52), and the triathlon (n = 18). The a priori intention was to clarify whether previously documented associations between the ACE polymorphism and elite athletes found in the study of a single sporting discipline could be duplicated in Russian athletes. Secondly, to determine whether any association would be lost if athletes from sports with different elements of power and endurance activity were combined. The athletes were stratified into groups according to event duration, covering a spectrum from the more 'power'-oriented to the more endurance-oriented. Short distance athletes (SDAs) consisted of 'sprinters' performing their given task in under 1 min; middle distance athletes (MDAs) were those competing over 1–20 min,

and long distance athletes were those performing over more than 20 min. The athletes were further classified by their standard of competition based on previous performances. 'Outstanding' athletes (n = 141) were at least national representatives and included 81 European and Russian champions and 19 Olympic or World champions. 'Average' athletes (n = 76) were regional competitors. Selection bias was reduced by employing multiple methods of recruitment.

Compared with a control group of 449 Caucasian Russians, the genotype distribution and allele frequency amongst the whole cohort (19.3% II, 51.2% ID, 29.5% DD, D-allele frequency 0.55) and that of outstanding athletes alone was similar to that amongst sedentary controls. In the cohort of outstanding athletes, ACE I/D polymorphism was indeed associated with event duration: an excess of D-alleles being noted in the SDA group, and an elevated frequency of the I-allele in the MDA group. There was no increasing linear trend of the I-allele with increasing aerobic component as has previously been described [20]. This may relate to differences in categorizing athletes. Myerson examined allele frequency by distance ran, whereas the study of Nazarov et al. [25] examined allele frequency by duration of event in order to compare outstanding athletes of different disciplines. As such, a 5,000 m runner or skier would be categorized as an MDA by the time criteria, but a long distance competitor by the distance criteria of Myerson and colleagues. Nevertheless, both groups (≥5,000 m and MDAs) show an excess of the I-allele, with similar frequencies (0.63 and 0.62, respectively).

These data support the hypothesis that the study of a mixed cohort of athletes from various sporting disciplines may obscure any association between elite athletes and the ACE I/D polymorphism. It is also important to note that if non-elite athletes are studied, association is often lost. The track and field athletes in the study of Nazarov et al. [25] demonstrated an excess of the D-allele in outstanding SDAs which, was not present in the average athletes. Similar findings have been demonstrated in other cohorts of athletes from different events. In a study of 126 Italian 'candidate Olympic athletes' when anaerobic-based and aerobic-based events were included together, there was no difference in genotype or allele distribution compared with a control group. However, although small numbers, comparing aerobic-based events (n = 33) with anaerobic-based events (n = 19) there was an excess of the II genotype in the former [26]. A further report in 139 Korean male elite athletes from various sporting disciplines (including basketball, soccer, baseball, gymnastics, volleyball, long-distance running, judo and ice hockey) also failed to show a difference in ACE I/D polymorphism frequencies compared with control (although there was a trend to excess of the I-allele in long distance runners) [27]. Indeed, if one looks back at the original study by Myerson [20] in UK Olympic athletes, it was also demonstrated that in combining 404 athletes from diverse disciplines such as judo and pole vaulting, there was no difference in allele frequency compared to the control population [20]. There has been however, the occasional report of an excess of the I-allele in mixed cohorts of athletes [28].

Subsequent studies examining cohorts of elite athletes from single sporting disciplines have also found an excess of the II genotype, for example in endurance runners versus soccer players [29] and Olympic class Spanish runners versus a control group [30].

That said, however, it has to be acknowledged that even in studies examining a single sporting discipline, associations have not always been found. The ACE I/D polymorphism was not found to relate to endurance athlete status in 221 national and 70 international Kenyan athletes [14]. However, the ACE I/D polymorphism only explains 13% of the variance in ACE activity in a general Kenyan population [14]. If the putative mechanism of effect is via differences in ACE activity and downstream effects within the RAS, then the degree of association between genotype and ACE activity could be crucial. Similarly, other studies have even found diametrically opposed results, for example a relative lack of the II genotype in rowers [31].

Although not strictly a single sporting discipline, few would argue that endurance is a critical factor in Ironman triathlons. In participants of the South African Ironman Triathlon a significantly higher frequency of the I-allele in the fastest 100 South African-born finishers (103 I, 51.5%, and 97 D, 48.5%) compared with the 166 South African-born control subjects (140 I, 42.2%, and 192 D, 57.8%) has been reported [32]. There was also a significant linear trend for the allele distribution among the fastest 100 finishers (I-allele = 51.5%), slowest 100 finishers (I-allele = 47.5%), and control subjects (I-allele = 42.2%; p = 0.033). Similarly, a study of 215 marathon runners found an increased frequency of the II genotype in the fastest runners [33].

It is important to realize that the ACE I/D polymorphism should not be considered a 'gene for human performance', but a marker that one would expect association only in the truly elite athlete according to the nature of the specific event. There has been an excess of the D-allele noted amongst sprinters (*D*-allele frequency 0.62 in those running ≤200 m) [20] and elite athletes completing their event in <1 min [25]. This relationship between the D-allele and power sports occurring over a short time period is distinct from that of the I-allele and enhanced endurance performance. If a discipline is spread over varying distances (such as swimming, running) then cohort stratification should be considered according to anaerobic/aerobic or power/endurance component.

Swimmers are a particularly interesting example. A strong relationship exists between upper body anaerobic power and performance in swim events from 50 to 400 m [34, 35] and upper body arm power is a highly significant performance factor in the longer swimming distances [35].

Accordingly, one study in European and Commonwealth championship Caucasian swimmers subdivided them into successful competitors over <400 and >400 m. A significant excess of the D-allele and DD genotype compared with large control groups was reported due to an excess of the D-allele and DD genotype in those swimming over the shorter distances [36]. A small study (n = 35) of elite very long distance swimmers classified as better at 1- to 10-km distances (n = 19) or those best at 25-km races (n = 16) again revealed an apparent excess of the D-allele in those better at

Table 1. Chronological summary of population association studies examining the relationship between ACE I/D polymorphism and human performance by study first author

Study first author	Subjects	Analysis by single sporting discipline?	Association with the ACE I/D polymorphism
Montgomery [1998]	25	yes, elite climbers	yes
Myerson [1999]	91	yes, runners	yes
Gayagay [1998]	64	yes, rowers	yes
Taylor [1999]	120	no, multiple disciplines	no
Karjalainen [1999]	80	no, multiple disciplines	no
Rankinen [2000]	192	no, multiple disciplines	no
Alvarez [2000]	60	no, multiple disciplines	yes
Woods [2001]	56	yes, swimmers	yes
Nazarov [2001]	141	no, but stratified by event duration	yes
Scanavini [2002]	126	no, multiple disciplines	no in whole cohort but yes in runners
Tsianos [2004]	35	yes, swimmers	yes
Oh [2007]	139	no, multiple disciplines	no
Lucía [2005]	27	yes, runners	yes
Collins [2004]	100	no (triathlon)	yes
Scott [2005]	291	yes, runners	no, but weak relationship between genotype and ACE activity in Kenyans
Hruskovicová [2006]	215	yes, runners	yes
Muniesa [2008]	141	no, multiple disciplines	yes, reduced I-allele in rowers
Juffer [2009]	52	yes, runners	yes

shorter distance swimming and an excess of the I-allele in those swimming ultra-endurance distances [37]. While a 1- to 10-km swim could never be considered a short event, perhaps the upper body arm power referred to by Hawley et al. [35] has primacy unless the event is a really long endurance swim. Sadly, the numbers are too small to reveal any resounding insight.

Myerson et al. [20] had also found a significant excess of the D-allele among elite swimmers. Interestingly, Nazarov et al. [25] found again that a mixed cohort of average and elite swimmers demonstrated no skew in genotype distribution but examining allele frequencies by duration of event in elite athletes only there was a significant excess of the D-allele in swimmers completing their event <1 min. Diluting a cohort with non-elite athletes may result in failure to find an association with the ACE I/D polymorphism. To test whether a genetic marker occurs more frequently in elite athletes than controls requires a homogenous cohort from the same sporting discipline with large, matched control groups (table 1).

The ACE I/D Polymorphism and Prospective Training Studies at Sea Level

To examine the effect of a gene polymorphism that may have only small, but significant, effects on human performance and to attempt to tease this effect out from the multifactorial make-up of an elite athlete often requires examining a period of gene-environment interaction. Most often, this involves studying the influence of a period of training on a performance phenotype.

The I-Allele and Endurance
The earliest prospective study examining the influence of the ACE I/D polymorphism on performance parameters was published 11 years ago. While perhaps not employing a gold standard method of assessing endurance, it did reveal a genotype-dependent difference in a performance parameter with training. Duration of loaded repetitive biceps flexion was independent of genotype at baseline in 78 British Army recruits but was found to increase 11-fold more amongst those of the II genotype compared to the DD genotype after a 10-week general physical training programme [38]. Similarly, in female non-elite Caucasian Turkish athletes over a 6-week training regimen, the II genotype related to better improvements in medium duration aerobic endurance performance (such that a significant linear trend: II > ID > DD occurred) [39].

A study in American recruits does not support these findings [40]. However, these authors specifically aimed to assess whether an association between the ACE I/D polymorphism and performance phenotypes with training existed in a heterogeneous, mixed-sex, multi-ethnic population of US military recruits. 147 recruits were studied before and after 8 weeks of basic training which, in marked contrast to UK recruits, is partly performed in streamed quartiles according to baseline fitness. In addition, the performance marker used was the Army Physical Fitness Test which the US Army itself considers to be a measure of combined endurance and strength. The application of a heterogeneous training programme to a heterogeneous cohort as might be expected did not reveal any significant ACE I/D polymorphism association. In addition, if the effect of the polymorphism is mediated by differences in ACE activity, it is important to remember that there is no such relationship in African-Americans, a significant portion of this cohort. It is more difficult to reconcile data from a study in Turkish Army recruits that found a performance enhancement after 6 months training related to the D-allele over a 2,400-metre running distance [41].

The D-Allele and Power-Oriented Performance
One report documents the effects of 9 weeks of strength training on quadriceps muscle strength in 33 healthy male volunteers [42]. A significant interaction between ACE genotype and isometric training occurred with greater strength gains shown by subjects with the D-allele. This increase in strength may contribute to the apparent excess of the D-allele among power-oriented events.

A further study subsequently has also demonstrated an apparent improvement in strength response to 6 weeks of training associated with the DD genotype in 99 Caucasian male non-elite athletes [43]. Similarly, in female non-elite Caucasian Turkish athletes over a 6 week training regimen, the DD genotype conferred a more advantageous performance enhancement in shorter duration and higher intensity endurance activities [39]. In support of these studies, although in 213 older obese people, an 18-month training programme of walking and light weight lifting resulted in individuals with the DD genotype showing greater gains in knee extensor strength compared with II individuals [44].

Although involving a shorter period of training, another cohort of elderly subjects showed that the DD genotype was associated with greater quadriceps muscle volume at baseline but did not influence their knee extensor muscle strength [45]. Peak elbow flexor strength in a large cohort of 631 subjects after training 2 days a week for 12 weeks was greater in the contralateral but not the trained arm in association with the DD genotype [46]. Other studies have found no improvement in elbow flexor strength associated with the ACE I/D polymorphism after training [47].

The ACE I/D Polymorphism and Human Performance at High Altitude

It was also noted over 10 years ago that in the extreme physiological environment of high altitude (HA) mountaineers demonstrate an allele skew compared to a normal population [20]. In 25 elite male British mountaineers who had ascended beyond 7,000 m without the use of supplemental oxygen there was a significant excess of the II genotype and I-allele with no DD genotypes in those climbers who had gone beyond 8,000 m without oxygen. This is an incredibly small group for a gene association study but we are limited to an extent by the relatively small numbers enduring such physiological stress. Again, despite the numbers, this report spawned a plethora of subsequent studies seeking to confirm or refute an association, and to examine potential mechanisms.

Subsequent work also reported a relative increased frequency of the I-allele (0.55 vs. 0.36, successful vs. unsuccessful, respectively) in those climbers who had reached 8,000 m, from a cohort of 139 who had attempted to do so [48]. Similarly, from a cohort of 284, success at climbing Mont Blanc (4,807 m) has been associated with the I-allele [49].

The ACE I/D Polymorphism and Human Performance: Potential Mechanisms Involved

The ACE polymorphism not only influences circulating ACE activity, it also appears to be a determinant of ACE at a cellular level [50–53]. This may have downstream

effects in the production of angiotensin II and breakdown of bradykinin that will influence various factors including blood flow, substrate utilization and cell growth relevant to human performance.

In support of this, DD subjects demonstrate greater plasma angiotensin II concentrations following angiotensin I infusion [54, 55], and the contractile response of internal mammary arteries to angiotensin I and II suggest that angiotensin I conversion is greatest in the presence of the D-allele [56]. Local tissue conversion of angiotensin I may also be relevant since DD subjects have a significantly enhanced forearm vasoconstrictor response to angiotensin I infusion that is not accompanied by differences in serum angiotensin II levels [57].

It is important to note that angiotensin II type 1 receptor (AT_1)-mediated angiotensin II stimulation is crucial for optimal overload-induced skeletal muscle hypertrophy [58]. Further, it has been demonstrated that AT_1 blockade in rats undergoing eccentric contraction training over 4 weeks prevents the training-induced increase in muscle mass and muscle contractile force compared with controls [59]. Angiotensin II is also known to increase the contraction-induced oxygen uptake and the tension during tetanic stimulation in rats [60]. This effect may come at a cost, and it is known that angiotensin II administration in rats reduces metabolic efficiency with skeletal muscle wasting [61]. The redirection of blood flow from type I muscle fibres to the type II fibres that are favoured in power performance (a sprinter may have 80% fast twitch fibres, an endurance athlete 20%) is also influenced by angiotensin II [60] and would be potentially beneficial in power-oriented events.

Other potential mechanisms by which varied angiotensin II levels could influence human performance are via its effect as a direct stimulator of cellular growth (both hypertrophic and hyperplastic); the induction of various endogenous growth factors and the facilitation of sympathetic nerve transmission by enhancing noradrenaline release from peripheral sympathetic nerve terminals and the CNS [reviewed in 62].

There is also evidence of reduced bradykinin degradation in II subjects with low ACE activity [54]. Reduced bradykinin degradation may favourably alter substrate metabolism in II subjects with improvements in the efficiency of skeletal muscle. Indeed, a bradykinin-mediated increase in nitric oxide production in skeletal muscle facilitates glucose uptake [63, 64]. Muscle work increases muscle blood flow (and bradykinin in human venous effluent) [65, 66] with increased glucose uptake in humans, an effect reproduced by bradykinin infusion in the human forearm that can be blocked by inhibiting bradykinin-generating proteases [67]. Further, flow-mediated vasodilatation has been reported to be ACE I/D polymorphism dependent with greater flow-mediated vasodilatation in II subjects compared to DD subjects [68]. This may represent the effect of an increased half-life of the vasodilator bradykinin associated with the I-allele and lower ACE activity as opposed to the blunted nitric oxide vasodilatory response seen in the forearm vessels of DD subjects [69].

It can be seen therefore that the effect of bradykinin on muscle blood flow and substrate utilization is a key factor. Indeed, the oxidative capacity of muscle (greatest

in the type 1 fibres that correlate with efficiency in cyclists) [70] is in direct correlation and may be interdependent with muscle kallikrein from which bradykinin originates [71].

In addition, a reduction in ACE activity leads not only to an attenuation of angiotensin II production and a decrease in bradykinin degradation but also to an increase in Ang-(1–7) [72]. This may further favour vasodilation and potential substrate delivery as the actions of Ang-(1–7) are most often opposite those of angiotensin II.

The important consideration however is whether differences in ACE, angiotensin II, bradykinin and Ang-(1–7) translate measurable factors that may be relevant in human performance.

A high level of aerobic fitness is an important requirement, but not a final determinant, of elite endurance performance. The standard measure of aerobic capacity, VO_{2max}, is influenced by genetic and environmental factors with marked inter-individual response to training. As such, in the search for a physiological link between the ACE I/D polymorphism and elite human performance, the study of central cardiorespiratory factors such as VO_{2max} was a natural avenue of investigation. As the most important environmental factor in determining VO_{2max} is regular physical activity, it is vital that any genetic study is charged with standardizing the training protocol within, and between, groups; however, despite several studies concluding that there has been no consistent effect of the ACE I/D polymorphism on VO_{2max} reported [40, 73, 74].

Hagberg et al. [75] did however find an increase in VO_{2max} associated with the I-allele that was entirely due to an increase in maximal arteriovenous oxygen difference (rather than increased in cardiac output). The effect of angiotensin II and bradykinin on local skeletal muscle bioenergetics may provide the key to unlocking a potential peripheral muscle effect rather than a central cardiopulmonary effect.

One report supports that notion. 58 British military recruits had their VO_{2max} and delta efficiency (delta work accomplished min^{-1} divided by delta energy expended min^{-1}, expressed as a percentage) assessed before and after 11 weeks of physical training [76]. VO_{2max} was no different before or after training according to ACE I/D polymorphism, but delta efficiency (independent of genotype at baseline) rose significantly with training only amongst those of II genotype (absolute change 1.87% for II subjects, –0.26% for DD), a proportional increase in efficiency of 8.62%. This would certainly be enough to have a significant biological impact. This study is supported by the greater peripheral tissue oxygenation and lesser rise in lactate, reflecting greater muscle efficiency, that occurs in II compared with DD subjects during exercise in patients with chronic airway disease [77] and the greater aerobic skeletal muscle efficiency found in Chinese chest patients with the II genotype [78], although not in 62 untrained sedentary females [79]. The study in military recruits has the advantage in maintaining other environmental factors as constants (subjects were sleeping in the same location for a similar duration, eating the same diet and exercising to identical supervised targets at identical times as well as being of one sex and from a very narrow age band), which helps to elucidate any subtle gene effect.

Subsequent work has offered further insight into this improvement in muscle efficiency. In 41 healthy young volunteers, skeletal muscle biopsies from the vastus lateralis have demonstrated that II subjects have a higher percentage of slow-twitch type I fibres (more efficient than fast-twitch fibres at low velocities of contraction). In addition, II subjects had a lower percentage of fast-twitch type IIb fibres than DD subjects with a linear trend for a decrease in type I fibres and increase in type IIb fibres from the II through the ID to the DD genotypes [80]. This may be a mechanism that explains the physiological link between the I-allele and improved endurance performance by means of improving muscle efficiency, and correlates with the fact that the oxidative capacity of muscle is greatest in type 1 fibres (that are more common in endurance runners and correlate with efficiency in cyclists) [70].

Possible Mechanisms Involved at High Altitude

Numerous studies have explored possible explanations as to why the I-allele may confer a performance advantage at HA. Obviously, a reduction in any acute mountain sickness (AMS) would be beneficial at HA. However, three studies have shown no apparent benefit related to the ACE genotype in terms of AMS, one cohort of 284 subjects [49], another of 151 [81] and another of 103 Nepalese [82]. Similarly, there is no genotype-dependent difference in HA pulmonary oedema [81, 83]. Other mechanisms have therefore been explored. One study examined an obvious factor, oxygen saturation, at HA [84]. Although again relatively small in subject numbers due to the nature of the activity, two groups of Caucasian subjects were studied. SaO_2 with ascent was significantly associated with the ACE genotype (SaO_2 at 3,870 m was 85.4, 87.5, 90.1% for DD, ID and II genotypes, respectively) in a rapid ascent group (n = 32) on Mount Everest with a relatively sustained SaO_2 in the II subjects. Whereas there was no similar association found with a slow ascent group on Kanchenjunga. This could possibly be explained by time-dependent altitude-related changes in the RAS.

This earlier work is supported by a more recent study in a Peruvian cohort [85]. In 142 subjects of mainly Quechua origin, resting and exercise SaO_2 was strongly associated with the ACE genotype at HA with approximately 4% of the total variance in SaO_2 being attributable to the ACE I/D polymorphism. II subjects maintained an SaO_2 around 2.3% higher than ID or DD subjects, regardless of whether the subjects were born at high or low altitude.

At altitude, diminished alveolar oxygen tensions lead to lower arterial oxygen tensions. However, the fall in oxygen availability with altitude is sensed by hypoxia-sensitive AT_1 receptors in the carotid bodies [86] which stimulate an increase in minute ventilation. This hypoxic ventilatory response helps sustain arterial oxygen saturations and tissue oxygen delivery and demonstrates a marked inter-individual variation. Patel et al. [87] have demonstrated from a cohort of 60 subjects that the hypoxia-induced percentage rise in minute ventilation is strongly and significantly associated with the II genotype (II 39.6 ± 4.1% vs. DD 28.4 ± 2.2%).

Conclusions

With the caveat of the frequent small cohorts that we must accept by the very nature of the elite groups being studied, there nevertheless remains a significant breadth and depth of evidence supporting a role for the ACE I/D polymorphism in human performance. There will never be a cohort of thirty thousand elite athletes from the same discipline. There will always be controversies with the interpretation of association studies. Attempting to reduce selection bias and giving due consideration to appropriate case definition as well as studying athletes from homogenous sporting disciplines with appropriately large control groups offer the best chance of reliable, reproducible outcomes. In concert with increasing data regarding an influence of the polymorphism on plausible mechanistic pathways, the balance of the evidence currently is in favour of there being a small but significant influence of the ACE I/D polymorphism on human performance. That small difference, following a suitable period of training and therefore gene-environment interaction, could be a significant factor between success and failure.

The ACE I/D polymorphism should not be considered a 'gene for human performance', but a marker of modulation such that one would expect an excess of the I-allele in the truly elite endurance athlete, with a concordant excess of the D-allele represented in the more power-oriented events. Of course, because elite athletes are still 'made' not 'born' there will always be elite athletes in endurance events with the DD genotype.

In what direction should the next generation of studies proceed? With particular reference to the association of the I-allele with elite endurance performance then a move from genetic to pharmacological studies could prove very revealing. If the low ACE activity associated with the I-allele has key physiological importance in the response to training, then studies using ACE inhibitor drugs to reduce the effect of ACE or angiotensin receptor blocker drugs (that only interfere with the action of angiotensin II and do not prolong the half-life of bradykinin) could provide great insight. Indeed, some commentators are already concerned that these drugs may, or have become, drugs of abuse in sport [88].

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α-Actinin-3 and Performance

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Abstract

The human sarcomeric α-actinins (*ACTN2* and *ACTN3*) are major structural components of the Z line in skeletal muscle; they play a role in the maintenance of sarcomeric integrity and also interact with a wide variety of structural, signaling and metabolic proteins. *ACTN2* is expressed in all muscle fibers, and expression of *ACTN3* is restricted to the type 2 (fast glycolytic) fibers that are responsible for forceful contraction at high velocity. There is a common stop codon polymorphism R577X in the *ACTN3* gene. Homozygosity for the R577X null-allele results in the absence of α-actinin-3 in fast muscle fibers with frequencies that vary from <1% in Africans to ~18% in Caucasians. A number of association studies have demonstrated that the *ACTN3* R577X genotype influences athletic performance in Caucasians; the frequency of the XX genotype is significantly lower than controls in sprint athletes, and it appears that α-actinin-3 deficiency is detrimental to sprint performance. In the general population, the *ACTN3* genotype contributes to the normal variations in muscle strength and sprinting speed. In an *Actn3* knockout mouse model, α-actinin-3 deficiency is associated with a shift in the characteristics of fast, glycolytic 2B muscle fibers towards a slow phenotype, with decreased muscle mass and fiber diameter, slower contractile properties, increased fatigue resistance, and an increase in oxidative enzyme activity. The shift towards a more efficient oxidative metabolism may underlie the selective advantage of the X-allele during evolution. In turn, the shift towards a 'slow' muscle phenotype in fast muscle fibers likely explains why loss of α-actinin-3 is detrimental to sprint performance.
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The Role of α-Actinins in Skeletal Muscle

The α-actinins are a family of actin-binding proteins that play a key role in the maintenance and regulation of the cytoskeleton [1]. In mammals, four α-actinin isoforms (α-actinin-1 to -4) have evolved from gene duplication events to fulfill similar functional requirements in different cell types [2–5]. *ACTN2* and *ACTN3* encode the skeletal muscle isoforms α-actinin-2 and α-actinin-3. *ACTN*2 is expressed in all skeletal muscle fibers (both fast and slow fibers), as well as cardiac muscle and the brain. *ACTN3* expression is restricted to type 2 (fast glycolytic) fibers in skeletal muscle,
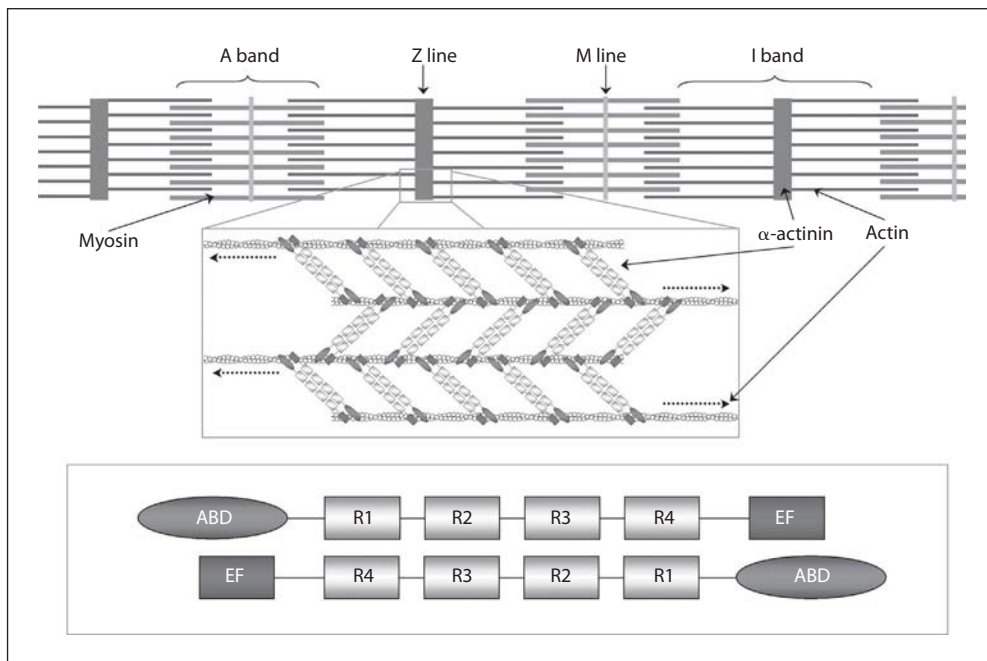
Fig. 1. Localization and domain structure of the sarcomeric α-actinins. The sarcomeric α-actinins are found at the Z line, where they anchor actin-containing thin filaments from adjacent sarcomeres. Antiparallel dimers of α-actinin cross-link actin filaments and stabilize them against force generated by the contractile apparatus (top inset; dashed arrows indicate direction of force). The lower inset illustrates the domain structure of the α-actinins and their antiparallel alignment in dimeric form. ABD = Actin-binding domain; R1–4 = spectrin-like repeats 1–4; EF = EF hand region. Adapted from MacArthur and North [44].

with low levels of expression in the brain [2]. The amino acid sequences of human *ACTN*2 and *ACTN*3 are 81% identical and 91% similar [2] and they form homodimers and heterodimers in vitro and in vivo, suggesting many interchangeable functions [6].

The contractile apparatus of skeletal muscle is composed of repeating units (sarcomeres) that contain ordered arrays of actin-containing thin filaments and myosin-containing thick filaments. The Z lines are electron-dense bands that run perpendicular to the myofibrils and anchor the thin filaments. The α-actinins are a major structural component of the Z line in skeletal muscle (fig. 1) and have a number of functional domains: an N-terminal actin-binding domain, a central rod domain, and a C-terminal region that contains two potential calcium-binding EF hand motifs [1]. The α-actinins provide a major multivalent platform for protein-protein interactions with many structural, signaling and metabolic proteins, resulting in diverse functional roles [7]. Based on their interaction with many of the structural and signaling proteins associated with the Z line, especially skeletal α-actin, the sarcomeric α-actinins (-2 and

-3) likely perform a static function in skeletal muscle to maintain the ordered myofibrillar array as well as a regulatory function in coordinating myofiber contraction [8].

The α-actinins interact with skeletal α-actin at the N-terminal and with other Z-line proteins (e.g. myotilin) at the C-terminal to organize and maintain the structure of the sarcomere in a three-dimensional lattice. The sarcomeric α-actinins are also thought to maintain muscle cell integrity by linking dystrophin (deficient in Duchenne muscular dystrophy) with the integrin adhesion system [9]. The interaction at the N-terminal with skeletal α-actin is regulated by binding of the phospholipid, phosphatidylinositol 4,5-biphosphate (PiP2) [10]. In vitro studies suggest that the C-terminus of sarcomeric α-actinins plays a crucial role in the maintenance of Z line integrity and myofibrillar organization [11]. The C-terminal region is the site of binding for a number of Z-line structural proteins, including myotilin [12] and a group of PDZ-LIM domain proteins such as ZASP and ALP [13]. Within the central repeat rod domain, the α-actinins interact with the Kv1.4 and Kv1.5 potassium channel proteins, fructose-1,6-bisphosphatase – a glycolytic enzyme [14], glycogen phosphorylase [15], and the calsarcins which bind to and regulate the expression of calcineurin – a signaling factor which plays a role in the specification of muscle fiber type, diameter and metabolism (oxidative versus glycolytic) [16]. Thus, differential expression of the sarcomeric α-actinins in different muscle fibers may influence their metabolic profile and/or fiber type specification (fig. 1).

α-Actinin-3 Deficiency Is Common in the General Population

The *ACTN3* gene contains a polymorphism, R577X, which results in conversion of the codon for arginine (R) at position 577 to a premature stop codon (X) [17]. This variation results in two versions of *ACTN3* in humans, a functional R-allele and a null X-allele. Homozygosity for the X-allele (XX genotype) results in complete deficiency of α-actinin-3 in humans [17]. The frequencies of the XX genotype differ in human populations ranging from ~1% in Eastern and Western Africans and South Africans, to ~18% in Europeans and up to ~25% in East Asians [18]. An estimated one billion people worldwide are deficient in α-actinin-3. The widespread deficiency of a structural protein which is highly restricted in expression in fast glycolytic fiber is very unusual, and raised the possibility that α-actinin-3 is functionally redundant, and that the closely related protein, α-actinin-2 is able to compensate for its loss in muscle. However, initial data suggested that *ACTN3* has function(s) independent of *ACTN*2 in human and mouse muscles [18]. The experimental evidence can be summarized as follows:

1 *ACTN3* is highly conserved during evolution. Sequence comparison of human, mouse and chicken *ACTN* genes suggest that human *ACTN2* and *ACTN3* diverged substantially earlier than 300 million years ago, and the proteins encoded by both genes have evolved very slowly since their divergence.

2 *ACTN2* and *ACTN3* are differentially expressed, spatially and temporally, during human and mouse embryonic development, and *ACTN2* expression does not completely overlap *ACTN3* in mouse postnatal skeletal muscle. *ACTN3* is the predominant fast fiber isoform in both mice and humans. There are ~30% mouse 2B fibers with no *Actn*2 expression, whereas *Actn*3 is expressed in all 2B fibers.

3 The marked variation in *ACTN3* XX genotype frequencies in human populations raised the possibility that the *ACTN3* R577X genotype confers a fitness advantage to humans under certain environmental conditions.

Subsequent studies in human populations, in elite athletes and in an *Actn*3 KO mouse model have confirmed that *ACTN3* is not functionally redundant, that *ACTN*2 cannot completely compensate for the loss of *ACTN3*, and that the presence or absence of α-actinin-3 influences human skeletal muscle performance. A detailed description of the experimental evidence is given in the following sections.

The Null X-Allele at the *ACTN3* Locus Has Undergone Recent Positive Natural Selection

The propagation of *ACTN3* deficiency in humans with marked variations in X-allele frequencies among different ethnic groups [17, 19] has raised the possibility that the X-allele has been positively selected in some populations. To explore the selective forces that have acted on the R577X polymorphism during human evolutionary history, Macarthur et al. [20] analyzed the DNA sequence base substitution rate and long-range linkage disequilibrium for recombination rate around R577X in individuals of European, East Asian and African ancestry. There was unusually low sequence diversity and high long-range linkage disequilibrium amongst X-allele-containing haplotypes compared with the R-allele in Europeans and Asians. This is consistent with strong, recent positive selection on X-allele in these populations – suggesting that the X-allele provided some sort of fitness advantage to modern humans adapting to the novel Eurasian environment [20].

ACTN3 Genotype Is Associated with Elite Athletic Performance

The force-generating capacity of glycolytic fast muscle fibers at high velocity and the capacity of the individual to adapt to exercise training are strongly genetically influenced, and contribute to the normal variation in muscle performance in the general population [21]. In addition, there appears to be an evolutionary predetermined 'trade-off' in performance traits for speed vs. endurance [22, 23] that is, an individual is inherently predisposed towards being more of a 'sprinter' or a 'stayer'.

α-Actinin-3 expression is restricted to fast muscle fibers and the R577X polymorphism has resulted in widespread α-actinin-3 deficiency in humans. This raised the
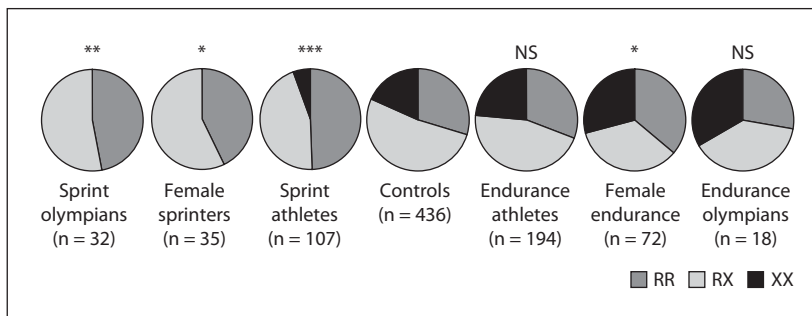
Fig. 2. Frequencies of the three *ACTN3* R577X genotypes in controls and elite athletes. The sample size in each group is shown. Female athletes and athletes who had competed at an Olympic level are shown as separate groups for both power/sprint and endurance athletes. Significant differences in genotype frequencies were seen between controls and total power athletes (p < 0.0001), female power athletes (p < 0.05), power Olympians (p < 0.01) and female endurance athletes (p < 0.05; χ^2 test for homogeneity of genotype frequencies between groups). Adapted from Yang et al. [24].

possibility that the *ACTN3* genotype contributes to normal human variation in skeletal muscle performance, and the presence or absence of α-actinin-3 may affect sprint or power activities performed by fast muscle fibers. Since any effect on muscle function will be most readily observable at the extremes of human performance, the effect of the *ACTN3* genotype has been most extensively studied in elite athletes.

Yang et al. [24] genotyped 301 elite Australian Caucasian athletes from 14 different sports and compared them with 436 healthy Caucasian controls. Athletes were defined as 'elite' if they had represented Australia in their sport at the international level; 50 of the athletes had competed at an Olympic level. There were considerable variations in *ACTN3* genotype frequencies between different sport groups of athletes, but there was no significant difference between genotype frequencies when comparing the athlete group as a whole with controls (χ^2 = 2.89, p = 0.06). The R577X genotype was then analyzed in a subset of 107 specialist sprint/power athletes and compared with a subset of 194 specialist endurance athletes. There were 32 power athletes and 18 endurance athletes who had competed at the Olympic level. Remarkably, all the sprint/power athletes had similar genotypic profiles with an extremely low frequency of XX (5% compared with 18% in controls) and a high frequency of RR (50% compared with 30% in controls (χ^2 = 19.70, p < 0.0001). The genotype effect was more marked in females and in sprint/power Olympians (25 male and 7 female), none of whom were XX (fig. 2). In contrast, elite endurance athletes had higher frequencies of the XX genotype compared with controls, although the effect was significant only for females (χ^2 = 6.15, p < 0.05). Importantly, genotype profiles in power and endurance athletes deviated in opposite directions and differed significantly from each other (χ^2 = 19.45, p < 0.001), explaining the lack of allelic association when the entire elite athlete cohort was compared with the controls [24].

Following this study, there have been five independent association studies performed in elite sprint/power athletes which confirmed the initial finding that the *ACTN3* XX genotype occurs at significantly lower frequency in sprint/power athletes [25–29]. However, several association studies have failed to substantiate a significant association between α-actinin-3 deficiency and improved endurance performance – although there is a trend apparent in one study [24]. The results of these studies are summarized below and in tables 1–3.

The association studies of the *ACTN3* genotype in sprint/power athletes have included various sporting disciplines such as gymnastics, skating, swimming, track and field, throwing and weightlifting (table 1). All these typical mixed sprint/power athlete cohorts demonstrated significant association with the *ACTN3* genotype, with reduced XX and excess RR genotype frequencies compared with the relative control populations. The consistency of these results provides supportive evidence for a detrimental effect of the XX genotype and beneficial effect of the RR genotype on sprint/power performance.

The effect of the *ACTN3* genotype on athletic endurance performance is not clear. The Australian study [24] (total 193 athletes) and Russian study [30] (total 456 athletes) examined a wide range of endurance sports, including cycling, rowing, swimming, track and field and cross-country skiing (table 2). Australian elite male endurance athletes had a slightly higher XX genotype frequency (19%) and similar genotype distribution to Australian control (XX 16%), while Australian female endurance athletes had a significantly reduced RR (20%) and *increased* XX genotype (30%) frequencies compared with the control (RR 30% and XX 20%; table 2). However, in Russian endurance athletes both males and females had significantly *reduced* XX genotype frequency (4–7% in athletes compared with 13–17% in controls).

To clarify these contradictory results, we reviewed all published association studies of specific endurance sports – road cycling, ironman triathlon, long-distance running and rowing (table 3). Again the results show inconsistencies. In an Australian cohort of 75 road cyclists, there was a significant increase in the XX genotype (30%) compared with the control (XX 18%). In a similar Spanish road cycling cohort (n = 50), there was a slightly higher XX genotype frequency (26%) but it was not statistically different from the control (XX 18%) [42]. The other endurance-specific sports studied to date include a large cohort of participants in two ironman triathlons, long-distance runners competing in distances ranging from 3,000 m to marathon and rowers competing at distances greater than 2,000 m. All were found to have similar *ACTN3* XX genotype frequencies to ethnically similar controls (table 3). The combined weight of evidence from these studies suggests that the *ACTN3* XX genotype has no consistent beneficial or detrimental effect on endurance performance.

Studies in African athlete and control populations suggest that α-actinin-3 deficiency is not a major influence on performance in African athletes [19]. The frequency of the X allele was extremely low amongst Kenyans and Nigerians (~1% XX genotype) and higher in Ethiopians (~11% XX genotype). The low baseline frequencies

Table 1. *ACTN3* R577X genotypes in Sprint/Power athletic performance

Origin	Gender	Athletes			Controls		p	Reference
		n	sports	genotype, %	n	genotype, %		
Australian	M	72	judo; speed skating; swimming; track and field; track-cycling	RR: 53 RX: 39 XX: 8	134	RR: 30 RX: 54 XX: 16	<0.001	24
	F	35	judo; track and field; swimming	RR: 43 RX: 57 XX: 0	292	RR: 30 RX: 54 XX: 16	<0.01	
Finnish	M and F	23	track and field	RR: 48 RX: 52 XX: 0	120	RR: 45 RX: 46 XX: 9	0.03	25
Greek	M and F	73	sprint and power athletes (jumping; decathlon; throwing; and track and field)	RR: 48 RX: 36 XX: 16	181	RR: 26 RX: 56 XX: 18	<0.02	27
	M and F	34	sprinters (track 100–400 m)	RR: 73 RX: 18 XX: 9	181	RR: 26 RX: 56 XX: 18	<0.001	
Russian	M	363	alpine skiing; artistic gymnastics; bodybuilding; figure skating; ice hockey; power lifting; ski jumping; soccer; speed skating; swimming; throwing; track and field; volleyball; weightlifting; wrestling	RR: 38 RX: 56 XX: 6	524	RR: 37 RX: 47 XX:16	<0.0001	29
	F	123	alpine skiing; artistic gymnastics; bodybuilding; figure skating; ice hockey; power lifting; ski jumping; soccer; speed skating; swimming; throwing; track and field; volleyball; weightlifting; wrestling	RR: 46 RX: 48 XX: 6.5	673	RR: 37 RX: 51 XX: 13	0.067	
Spanish	M	60	soccer players	RR: 48 RX: 37 XX: 15	123	RR: 29 RX: 54 XX: 17	0.041	28
USA	M and F	75	bodybuilding; powerlifting; and college level strength	RR: 31 RX: 63 XX: 7	876	RR: 38 RX: 46 XX: 16	0.005	26

p value refers to the difference in XX genotype frequencies between athlete population and controls.

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Table 2. *ACTN3* R577X genotype in endurance athletic performance

Origin	Gender	Athletes			Controls		p	Reference
		n	sports	genotype, %	n	genotype, %		
Australian	M	118	cycling; rowing; swimming; track and field; XC skiing	RR: 28 RX: 53 XX: 19	134	RR: 30 RX: 54 XX: 16	NS	24
	F	75	cycling; rowing; swimming; track and field; XC skiing	RR: 20 RX: 50 XX: 30	292	RR: 30 RX: 50 XX: 20	<0.05	
Russian	M	293	biathlon ≥15 km; race walking ≥10 km; road cycling ≥50 km; rowing ≥2 km; swimming ≥0.8 km; triathlon; XC skiing ≥10 km	RR: 40 RX: 53 XX: 7	532	RR: 36 RX: 47 XX: 17	<0.01	30
	F	163	biathlon ≥15 km; race walking ≥10 km; road cycling ≥50 km; rowing ≥2 km; swimming ≥0.8 km; triathlon; XC skiing ≥10 km	RR: 37 RX: 59 XX: 4	679	RR: 37 RX: 50 XX: 13	<0.01	

p value refers to the difference in XX genotype frequencies between athlete population and controls.

of the three populations tested mean that any associations with sprint performance would likely be obscured. In Ethiopians, who excel in endurance sports, there was no increased frequency in the XX genotype in endurance athletes compared with controls.

Mechanisms Underlying the Effects of α-Actinin-3 Deficiency on Muscle Performance – Studies in *Actn3* KO Mouse and in Non-Athlete Humans

In order to understand the possible mechanisms underlying the effects of α-actinin-3 deficiency on muscle structural and physiological properties, we will now review studies in the α-actinin-3 knockout (*Actn3* KO) mouse model, and compare them with studies that have been performed to date in non-athlete human populations.

The *Actn3* KO mouse mimics human *ACTN3* deficiency in many ways [20]. The KO mice are morphologically indistinguishable from their WT littermates on gross physical inspection and general autopsy; they showed normal ultrastructure of Z line and actin thin filaments at the light and electron microscope level; they did not demonstrate substantial loss of fast (2B) fibers as defined by staining for myosin heavy

Table 3. ACTN3 R577X in specific endurance sports

Origin	Athletes			Controls		p	Reference
	n	sports	genotypes, %	n	genotypes, %		
Australian	71	Ironman triathlon	RR: 34 RX: 48 XX: 18	436	RR: 30 RX: 52 XX: 18	NS	Yang et al., unpubl.
	75	road cycling	RR: 31 RX: 39 XX: 30	436	RR: 30 RX: 52 XX: 18	<0.05	24
	84	rowing	RR: 32 RX: 47 XX: 21	436	RR: 30 RX: 52 XX: 18	NS	
Finnish	40	long-distance running	RR: 50 RX: 40 XX: 10	120	RR: 45 RX: 46 XX: 9	NS	25
Italian	42	rowing	RR: 21 RX: 60 XX: 19	102	RR: 31 RX: 27 XX: 22	NS	40
South African Caucasian	457	male Ironman triathlon	RR: 35[1] RX: 45[1] XX: 20[1]	143	RR: 27[1] RX: 52[1] XX: 21[1]	NS	41
Spanish	52	long-distance running	RR: 25 RX: 58 XX: 17	123	RR: 28 RX: 54 XX: 18	NS	42
	50	road cycling	RR: 28 RX: 46 XX: 26	123	RR: 28 RX: 54 XX: 18	NS	
	39	rowing	RR: 33 RX: 44 XX: 23	123	RR: 28 RX: 54 XX: 18	NS	43
Ethiopian	75	long-distance running	RR: 46 RX: 46 XX: 8	100	RR: 44 RX: 44 XX: 12	NS	19
Kenyan	284	long-distance running	RR: 65 RX: 24 XX: 1	158	RR: 84 RX: 15 XX: 1	0.036	

[1] Genotype frequencies were estimated from figure 1 in publication [41].

chain isoforms. Unlike humans, α-actinin-2 is not normally expressed in all fast fibers in WT mice [18]. In *Actn3* KO mice, however, α-actinin-2 is upregulated and expressed in all fiber types, similar to the expression pattern seen in human muscle [20]. Thus, as in humans with the *ACTN3* XX genotype, α-actinin-2 is the only sarcomeric α-actinin expressed in all fiber types.

Muscle Metabolism
The *Actn3* KO mouse muscles showed more intense staining than those from WT littermates for two markers of aerobic metabolism, NADH-tetrazolium reductase and succinate dehydrogenase [20]. Further quantitative analysis of enzymes demonstrates significant decreased activity in the anaerobic glycolytic pathway and increased activity in the aerobic oxidative pathway [20, 31]. Specifically, the level of anaerobic enzyme lactate dehydrogenase was reduced by 16% in KO muscle. The mitochondrial enzymes, citrate synthase, succinate dehydrogenase and cytochrome c oxidase, were increased by 22–39%. Two enzymes involved in fatty acid oxidation, β-hydroxyacyl-CoA dehydrogenase and medium chain acyl-CoA dehydrogenase, also showed 30–42% higher levels in *Actn3* KO muscles. These results suggest a shift in muscle energy metabolism in *Actn3* KO mice towards the more efficient aerobic/oxidative pathway.

Fiber Contractile Properties
Physiological studies in isolated extensor digitorum longus (fast twitch) muscles from KO and WT mice, displayed longer twitch half-relaxation times and better recovery from fatigue and enhanced force recovery following fatigue in KO compared with WT [20]. These findings further support the shift of properties of fast-twitch muscle in KO mice towards the characteristics of slow-twitch muscle. As a consequence, *Actn3* KO mice are able to run a significantly longer distance than the WT littermates before reaching exhaustion [20].

Muscle Strength and Power
α-Actinin-3 deficiency results in subtle but significant decrease in muscle strength in both mouse and human – although it must be noted that muscle strength in α-actinin-3-deficient mice and humans is still within the normal range.

  The grip strength in *Actn3* KO mice is 7.4% lower in male ($p = 0.007$) and 6.0% lower in female ($p = 0.02$) compared with the WT littermates [31]. In non athlete human populations, there have been several studies demonstrating the muscle strength difference between *ACTN3* genotypes. Women with the XX genotype had lower baseline elbow flexor isometric (MVC) strength compared with individuals with RR genotypes ($p < 0.05$) in an adult cohort (n = 355) [32]. In a study of fast-velocity knee extension strength in a group of young men (n = 90, 18–29 years), relative dynamic quadriceps torques at 300°/s was lower in XX individuals ($p < 0.05$) [33]. In another female cohort with a wide age range (n = 394, 22–90 years), the knee

extensor concentric peak torque (p = 0.019) and eccentric peak torque (p = 0.022) were lower in women with the XX genotype [34].

In a study of Greek adolescent boys (n = 525), XX individuals took significantly longer time to complete a 40-meter sprint (average time: XX = 6.13, RX = 6.00, RR = 5.92, p = 0.003) [35]. Interestingly, sprint times for individuals with the RR genotype were significantly faster than heterozygotes (RX), who were, in turn, significantly faster than XX – raising the possibility of a 'co-dominant' effect of the R and X allele – resulting in a 'dosage' effect relative to the amount of α-actinin-3 present in fast fibers. The potential heterozygote effect of the *ACTN3* genotype has not yet been studied in any detail in either mice or humans.

α-Actinin-3 deficiency also appears to affect strength and power performance at the extremes of fitness. Within a group of human elite male road cyclists (n = 46), individuals with XX-genotypes were found to have less peak power output (p = 0.035), less power to tolerate high submaximal workloads (p = 0.029~0.01) compared with those with RR genotypes [36]. In a study to assess the genotype effect on ageing progression over a 5-year period, old women (70–79 y) with XX genotypes had a ~35% greater risk to develop difficulties in walking and climbing stairs compared with those with RR genotypes, and old men (70–79 years) with XX genotypes had greater increase in times to complete 400-meter walking [37]. Recently, the *ACTN3* genotype has been shown to influence exercise capacity in women with the metabolic myopathy, McArdle's disease; those with the X allele had a higher VO_2 peak and a higher oxygen uptake (VO_2) at the ventilatory threshold than RR homozygotes [38].

Muscle Mass, Fiber Diameter and Fiber Type Proportion
Actn3 KO mice were shown to have a reduction in lean muscle mass using dual-energy X-ray absorptiometry scans [31]. This correlates with a decrease in the weight of individual muscles harvested from KO and WT mice. All muscles that contain fast-twitch fibers (and hence express α-actinin-3), including extensor digitorum longus, medial biceps, triceps, tibialis anterior, gastrocnemius, quadriceps and spinalis thoracis, have significant mass reduction in KO compared with WT [31]. Interestingly, a study in humans (n = 394) using a dual-energy X-ray absorptiometry scan also found a significant reduction in lean mass (p = 0.04) as well as fat mass (p = 0.02) in women with the XX genotype compared with women with combined RR and RX genotypes [34]. In this cohort, body mass index of women with the XX genotype was also significantly smaller than women with the RR genotype (p = 0.013) [34]. In a study examining the cross-sectional area (CSA) of human thigh muscle, postmenopausal women with the XX genotype had significantly reduced CSA compared with women with the RR genotype (p < 0.05) [39].

The reduction in muscle mass in *Actn3* KO mice is attributable to a specific decrease in fiber diameter of fast twitch (2B) muscle fibers – again reflecting a shift towards the phenotype of slow-twitch (type 1) fibers [31]. There was no difference in the diameter of type 1 or 2A fibers where α-actinin-3 is not usually expressed. There

is tantalizing data – albeit limited by small study numbers – to suggest that the presence or absence of α-actinin-3 influences fiber diameter and fiber type in humans. In a cohort of young men (18–29 years), muscle biopsies were compared between 22 XX and 22 RR individuals. The percentage surface area and number of type 2X fibers (equivalent to 2B fibers in the mouse) were significantly less in those with the XX genotype compared with RR, while there were no significant differences in the size or number of type 1 and 2A fibers [33].

Conclusions

The *ACTN3* R577X polymorphism results in a well-defined phenotype (presence or absence of a muscle structural protein), and has a biologically plausible effect on skeletal muscle performance in athletes and in the general population; that is, loss of α-actinin-3 from fast-twitch muscle fibers is detrimental to sprint and power activities. The *Actn3* knockout mouse model provides mechanistic insights into the effects of the *ACTN3* genotype in humans. Loss of α-actinin-3 in mouse results in a shift in a variety of properties of fast fibers towards those characteristic of slower fibers. The slower contractile properties, reduced fiber diameter and decrease in anaerobic metabolism associated with α-actinin-3 deficiency explains the detrimental effect on sprint and power performance observed in multiple Caucasian athlete association studies. The shift in muscle metabolism toward the more efficient aerobic pathway may have provided adaptive benefits during a period of human evolution when man migrated out of Africa and faced famine and cold; perhaps explaining the strong positive selection for the X allele in Europeans and Asians. The X allele is much rarer in African populations (XX genotype frequencies in most African populations are less than 1%). Thus, the *ACTN3* genotype is unlikely to be an important determinant of variations in human performance in African populations. Whether or not α-actinin-3 deficiency influences adaptations for endurance performance is less clear; there may be more subtle effects that remain to be explored such as the effect on response to exercise and training.

Variations in the *ACTN3* R577X genotype may also have public health implications; an estimated one billion people worldwide are deficient in α-actinin-3. The reduced muscle mass and strength associated with α-actinin-3 deficiency in the general population is within the range of normal human variation. However, α-actinin-3 deficiency could modify quality of life and severity of muscle weakness at the extremes of human fitness – for example, in the less mobile ageing population prone to muscle sarcopenia, in patients with cachexia due to chronic illness and in patients with muscle disease. Intriguingly, the alterations in muscle metabolism associated with the *ACTN3* genotype could modify response to diet and the risk of developing common disorders in the modern world such as type 2 diabetes and obesity. Future studies of the advantages and disadvantages that the ACTN3 X-allele has conferred on humans

will provide interesting insights into how genetic factors that influenced survival in ancient humans contribute to performance and health in modern man.

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East African Runners: Their Genetics, Lifestyle and Athletic Prowess

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Abstract

East African runners have dominated distance running events for over 5 decades. Some explanations have been advanced to explain why such a small population has dominated distance running events over time. Suggested reasons include, among others, a genetic predisposition, diet, living at high altitude as well as sociocultural background. This chapter gives possible insight into the past, present and hopefully future success of East African runners; it mainly explores the foundations of running excellence, talent identification, diet and injury management methods used by East African runners. The chapter also explores means and ways by which East African runners can sustain their running excellence by using their past experiences, to perfect the present and predict the future.

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The continued excellence of East African athletes in distance running events continues to produce a lot of debate and interest across the globe. In fact, there has been an overabundance of opinions and theories aimed at explaining the phenomenal athletic prowess of the 'East African running machines – the exquisite runners'. Authors have suggested a superior genetic predisposition, sociocultural upbringing and sound nutrition as some of the possible factors behind the success of East African runners [1–7]. Runners from the eastern part of Africa nevertheless continue to enjoy unrivalled success in distances ranging from 800 meters to the marathon [8]. This chapter gives an academic discourse of the role that possible candidate genes coupled with habitual lifestyle could have lead to the success of East African runners.

Genetics of Performance and the East African Runners

The genetic predisposition (nature) of *Homo sapiens* plays an important role in determining many characteristics including ability to succeed in athletics and sports. Our

diet, upbringing and habitual physical activity also play a large role in enhancing and developing our athletic potential. One may, however, have the genetic potential to become a world-class athlete, but if one has not been nurtured to develop athletic excellence then one is unlikely to achieve that potential [9]. It is, however, worth noting that our genes may also limit our performance [9, 10].

Genetics have a large influence over many attributes necessary for athletic excellence such as strength, muscle size and muscle fiber composition (fast or slow twitch), anaerobic threshold, lung capacity, flexibility [11]. An athlete's cardiac capacity, or the heart's ability to deliver enough oxygen to the working skeletal muscles, for example, is a major determinant in endurance athletics. This too, is largely determined by genetics.

Research on aerobic endurance has shown that some people respond more to training than others [12]. Therefore, even if one has a low genetic potential for endurance, one may respond well to training and develop one's potential more completely than someone with a genetic 'talent' who does not respond to training. Training also increases cardiac efficiency, but the extent of this increase may be determined genetically [12].

It is hypothesized that great runners such as those from the eastern part of Africa are endowed with 'running' genes [7]. Several studies have been conducted to date to elucidate the role of genes in sports performance. Some studies have for example showed a significantly lower frequency of the 577XX (α-actinin-3-deficient) genotype in sprint or power athletes compared with controls, suggesting that the presence of wild-type α-actinin-3 enhances performance in sprint-type activities and an increase in the frequency of the 577XX genotype has been documented in some, but not all, endurance athletes [13, 14].

The role of the ACE gene, one of the most widely studied genes in recent times, has also been implicated in athletic performance [10, 15]. A study by Collins et al., [16] found a significantly higher frequency of the I-allele of the ACE gene within the fastest 100 South African-born finishers compared with the 166 South African-born control subjects. Myerson et al. [17] found an increasing frequency of the I-allele with distance running. A study investigating the association of DNA sequence variants within the ACE, angiotensinogen and angiotensin receptor type 1 genes with 60 elite (professional) athletes (25 cyclists, 20 long-distance runners, and 15 handball players) and 400 healthy controls showed that the I-allele occurred at a significantly higher frequency in athletes compared with controls [18].

Scott et al. [19] assessed the association between ACE gene variations and elite endurance athlete status in an African population successful in distance running. DNA samples were obtained from 221 national Kenyan athletes, 70 international Kenyan athletes, and 85 members of the general Kenyan population. Previous findings that many of the most successful East African athletes reside and train at altitude [4, 20] may have supported a role for the ACE gene in the determination of their success in distance running. Many Kenyan athletes live and train at altitudes of over 2,500 m

[6]. It is therefore conceivable that there has been selection for the I-allele of the ACE gene amongst such a population which may contribute to the altitude tolerance and endurance performance phenotypes. Results from the study by Scott et al. [19] did not support this hypothesis as there was an absence of an association between either I/D or A22982G polymorphisms within the ACE gene and elite Kenyan athlete status. For the East African group, the ACE gene therefore probably does not contribute significantly to the phenomenal success of Kenyan endurance runners in international distance running competition.

In another study, Yang et al. [21] looked at the ACTN3 R577X polymorphism among East and West African athletes. The R577X polymorphism was genotyped in 198 Ethiopian controls and 76 elite Ethiopian endurance athletes, 158 Kenyan controls and 284 elite Kenyan endurance runners, and 60 Nigerian controls and 62 elite Nigerian power athletes. The study found no significant deviations from the Hardy-Weinberg equilibrium in the populations tested. The XX (α-actinin-3 null) genotype was absent in Nigerians, had an extremely low frequency among Kenyans (about 1%), and a higher frequency in Ethiopians (about 11%). The study, therefore, did not support the hypothesis that the ACTN3 null X-allele contributes to the success of the East African endurance runners relative to their source populations. In addition, the extremely low XX genotype frequency in the Nigerian control population (0% in this study) ruled out the possibility of detecting a lower frequency of the XX genotype in Nigerian power athletes.

In a related study, Scott et al. [22] compared the frequencies of mtDNA haplogroups found in elite Kenyan endurance athletes with those in the general Kenyan population. DNA samples were obtained from 221 national level Kenyan athletes (N), 70 international Kenyan athletes (I), and 85 members of the general Kenyan population (C). mtDNA was classified into haplogroups by sequencing 340 bases of hypervariable sequence (HVS I), and by genotyping known restriction sites. Frequency differences between groups were assessed using exact tests of population differentiation. Results showed that the haplogroup distribution of national (p = 0.023) and international athletes (p < 0.001) differed significantly from controls, with the international athletes showing a greater proportion of L0 haplogroups (C = 15%, N = 18%, I = 30%), and lower proportion of L3* haplogroups (C = 48%, N = 36%, I = 26%). Although many of international athletes originated from the Rift Valley province in Kenya relative to controls (C = 20%, N = 65%, I = 81%), this province did not differ in haplogroup distribution from other regions (p = 0.23). Similarly, although Nilotic ethnicities were overrepresented amongst the athletes (C = 21%, N = 60%, I = 79%), haplogroup distributions did not differ between Bantu and Nilotic subjects (p = 0.12). The study concluded that International athletes differed in their mtDNA haplogroup distribution relative to the general Kenyan population. They displayed an excess of L0 haplogroups, and a dearth of L3* haplogroups. These findings are consistent with the idea that certain mtDNA haplogroups are influential in elite Kenyan distance running, although population stratification cannot be ruled out.

The Inspiration of Young Runners from East Africa

There are a number of reasons that can account for the motivation and inspiration of young and upcoming runners from East Africa [8]. These include and are not limited to:

1 Exposure to running at a young age,
2 Contact with training groups, which include international athletes, in local areas,
3 The presence and freedom to mix and mingle with role models,
4 Opportunities to train alongside an Olympian or World Champion,
5 Athletics is viewed as a 'gateway to fame and riches'.

The Diet and Way of Life of East African Runners

Nutritional supplements are still largely unknown among most East African athletes. The staple diet of *'ugali'*, accompanied by traditional vegetables *'isochot','osuga'* and *'isakiat'* are considered good for energy. *Ugali* also sometimes called *sima* or *sembe* is a cornmeal product and a staple starch component of many African meals, especially in East Africa. It is generally made from maize flour (or ground maize) and water, and varies in consistency from porridge to a dough-like substance.

The drinking of milk and blood is still common among some communities in Kenya, especially the Kalenjin and Masai. Breakfast consists of *'uji'* – a porridge made of millet. There is for example no 'junk food', no chocolate, and no ice cream in Kenyan villages. Children living in these villages are also not aware of these foods. A study by Onywera et al. [20] on the dietary habits of 12 elite Kenyan runners for a 7-day period found that the estimated energy intake (EI) over the 7-day assessment period (2,987 ± 293 kcal) was significantly lower than estimated (using PAR) energy expenditure (EE; 3,605 ± 119 kcal; $p < 0.001$). This is also reflected by the low EI:EE ratio (0.85 ± 0.08). The average daily EI of the runners was approximately 620 kcal (17%) below the average estimated daily EE for the athletes.

Most young athletes in East Africa run barefooted. In fact, shoes are still a novelty in most parts of Africa. We cannot forget Christopher Kosgei during the Grand Prix meets in Europe and Peter Chumba who won two gold medals during the first World Juniors Championships in Athens in 1986 bare feet. The entire junior women's gold medal winning team at the 1993 World Cross Country Championships in Spain competed bare foot. It is believed that direct contact with the hard ground strengthens the feet of the runners right from an early age. Although injuries are inevitable in any sporting event, East Africans have their own traditional way of managing them. Some of the traditional remedies for injuries include:

1 Bathing wounds in salty hot water,
2 Applying the cream of the milk to remove a thorn,
3 The Kalenjin tribe rubbing the leaves of the *'irokwet'* tree to a bruise.

The only cure that is used by many runners especially in the rural areas for stiff muscles or soreness is rest or rubbing Vaseline, animal fat, the bark or leaves of certain trees on the stiff or sore muscles.

Athletics Facilities before 'Westernization'

During the time preceding the 1980s, few schools in Kenya could afford their own track and none had a gymnasium or indoor facility. Athletes trained on the dirt tracks and pathways. They also used crude and/or improvised equipment [8, 23]. Today, many still refer to the javelin as a 'spear' and the shot as 'the stone' – a reference to what was used several years ago. Stopwatches were a novelty for many until recently. Few athletes knew their times or splits. We are fairly sure that there are few or no stadium records in Kenya. The idea of electronic timing is still a dream except, perhaps, at the national senior championships or trials. For the purpose of weight training, specific exercises and monitoring equipment, schools like St. Patrick's High School located in Iten at 8,000 feet above sea level, had to do with improvisation and guesswork. It is worth noting that St. Patrick's High School has, over the last 30 years, produced world-class long distance athletes. Some of the alumni include Ibrahim Hussein, winner of three Boston Marathons and one New York City Marathon; Peter Rono, a 1988 Olympic gold medalist at 1,500 m; Wilson Boit Kipketer, a 1997 world champion and 2000 Olympic silver medalist in the 3,000-meter steeplechase. Athletes used 'homemade' weights with little reference to accuracy or safety. Heart rate monitors, treadmills, let alone GPS locators, are items that are only read about in books [8].

Advantages of Such a Situation

The above scenario had some advantages. It prevented coaches and trainers from setting limits to workouts and programs, from measuring and waiting to be told by a machine what you already knew through common sense and instinct. Do you need an instrument to tell you that you're training too hard or that you're tired? Or that you have an injury to a knee? Learning to listen to their bodies and how the athletes feel was and still is an important part of a monitoring and evaluating process in Kenya. St. Patrick's for example has no track or gymnasium. They depend on a dilapidated stadium about 4 km away from the school, with a poorly maintained dirt track which becomes muddy during the rainy season. They have to make full use of the open countryside, its quietness and isolation, the closeness of nature, the dirt roads, the hills and forests.

Talent Identification and Nurturing

Most coaches often stand on the sideline during a primary schools athletics competition and wonder if someone out there has the potential to be an Olympic champion. They identify talented runners based on the 'theory of probability'. This takes into account the interest, personality, background, education, body structure, posture, movement, running style, pace, body size and shape, muscular structure and alignment of a particular runner. Br. Colm says: 'Having had little or not technical background to coaching athletics, I depended very much upon my ability to observe and learn from the athlete. There was an element of "trial and error" for me and, to some extent, still is.' [Cornnel, pers. commun., 2004].

The Role of Parents in Athletics Nurturing in East Africa

For many years, parents played little or no role in athletic development of their children. Many parents still consider running as a pastime or entertainment for youngsters rather than a profession. Some may feel that their children could have 'better things to do'. We encourage parents to be part of their children's athletic development. This is especially true of 'the girl athlete'. Traditionally girls were married off for dowry, they had their role in the home and their place was in the kitchen. An athletic career had little to do with it. When identifying a girl athlete – we ensure that parents and family understand the implications and give support to their daughter. Married women in Kenya were almost banned from competitions in the 1960s and 1970s. It was taboo for a married lady to dress in shorts and vest with bare legs and arms. Although girls had represented Kenya at major competitions, there is not yet a female 'running culture'.

The Present Scenario with Regard to Training and Preparation of Athletes

Things have not changed much especially with regard to training and preparation of athletes for local and international competitions. Many athletes still 'listen' to their bodies during training and competitions. Most of the athletic coaches are either former athletes themselves or officers working for the armed forces or police. Crude methods of training and monitoring of performance are still very popular.

Kenyan athletes have stuck to the past ways of living and training. They still cherish the lives lead by the likes of Mike Boit, Abebe Bikila, Kipchoge Keino and Amos Biwott among other pioneer runners. Most African athletes and coaches have not appreciated the role and place of science in boosting their performance and excellence on the track and in the field. As illustrated in figure 1, Onywera et al. [6] noted that Kenyan distance runners are motivated by economic reasons
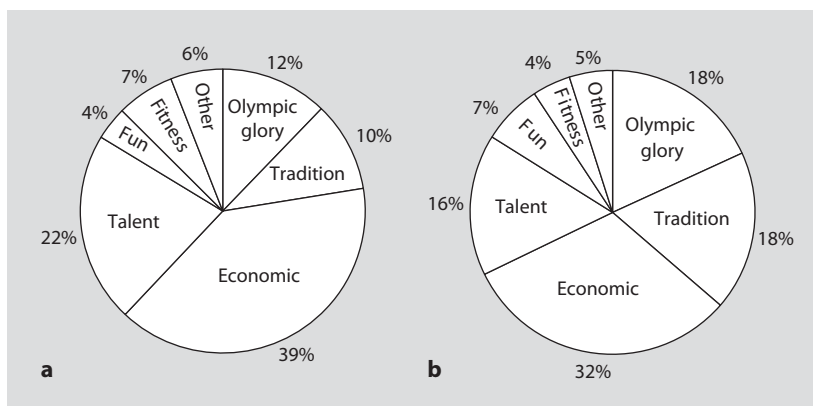
Fig. 1. Motivation to become a competitive athlete. The national (**a**) and international (**b**) athlete groups differed significantly from each other.

although 18% of international athletes, especially those who ran in the 1970s and 1980s, ran for Olympic glory.

Poverty and unemployment are major cancers in most African countries. It would be understandable, therefore, that economic reasons act as an important contributing factor to the success of East African athletes in distance running, a factor not accounted for in any other studies to date.

Conclusion

Having looked at various studies on the genetics of human performance as well as the way of life of East African runners, it is clear that more research needs to be done to unravel the mystery that is East African running excellence. More studies and resources are needed to explain the phenomenal success of East African runners.

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Gene-Lifestyle Interactions and Their Consequences on Human Health

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Abstract

Our genes are the conduit through which the environment communicates to the cells in our bodies. The responses to these signals include hormonal, metabolic and neurological changes to tissues and organs that manifest as phenotypes – measurable responses to gene transcription and translation. Thus, the health consequences of lifestyle behaviors such as physical activity, which can be broadly defined as 'environmental' exposures, are channeled through our genes. The extent to which these signals are conveyed depends in part on the structure and function of our genome. Hence, even when exposed to the same exercise regimes or doses of physical activity, responses vary markedly from one person to the next; some experience marked changes in disease phenotypes such as lipid and glucose concentrations, adiposity, or blood pressure levels, whilst others appear unresponsive. It is this process that underlies the concept that we will discuss in this chapter, a concept termed gene-lifestyle interaction. The aims of this chapter are (a) to convey to the reader the fundamental principles of gene-lifestyle interaction; (b) to describe the historical basis to this area of research; (c) to explain how understanding gene-lifestyle interactions might enhance our knowledge of the molecular mechanisms of disease; (d) to speculate on the ways in which information of gene-lifestyle interactions might eventually facilitate disease prevention, and (e) to overview the published literature which has focused on obesity as an outcome.

Obesity and type 2 diabetes are major global health concerns owing to their rapidly increasing prevalences and the severe burden they impose on affected individuals and healthcare systems. Both diseases share behavioral and genetic determinants, with obesity frequently preceding the onset of diabetes. Genome-wide association studies (GWASs) have spearheaded a major transition in the discovery of the genes that predispose to obesity and diabetes. For almost all common complex diseases, the number of confirmed genetic risk variants has increased manifold in the past 3 years. Prior to 2006, only a handful of polymorphisms had been equivocally associated with type

2 diabetes or common obesity [1]. Today, the identities of roughly 40 independent diabetes or obesity-predisposing loci are known, most of which have been discovered using GWASs. These discoveries have rejuvenated interest in the role of genetics in personalized medicine. However, the successful translation of this information into the clinical setting is predicated on two assumptions; the first is that the level of risk conveyed by a genetic variant is clinically meaningful and the second is that, if so, genetic risk can be offset through clinical intervention. To this end, the notion that prescription exercise regimens might one day be tailored to the genotype of the patient is becoming increasingly popular [2].

The purpose of this chapter is to introduce the reader to the concept of gene × environment interaction (also known as context-dependent genetic risk), to describe how genetic variants and lifestyle behaviors interact, and to provide a perspective on how this information might one day facilitate public health interventions to prevent or delay the onset of cardiometabolic diseases.

For much of the past 100,000 years, humans were hunter-gatherers, where the acquisition of food necessitated physical activity and where exposure to the extremes of heat or cold was common. These demands on energy metabolism imposed selective pressures that favored the survival and reproduction of lineages that were genetically enriched to thrive in physically demanding environments. By contrast to the vast majority of human evolution, the past century has witnessed a progressive decline in habitual physical activity levels and a relative overconsumption of foods rich in sugar and animal fats. Thus, for many of us, our lifestyles are massively disconnected from those which were common throughout evolution – those within which our genomes are designed to function. For many indigenous populations hailing from ancestral lines defined by migration, famine, and cold exposure, the genomic advantage gained throughout human evolution may predispose them to a much higher risk of developing metabolic and cardiovascular diseases when living modern industrialized lifestyles. This may help explain the high level of variation in the rates of obesity and type 2 diabetes across populations living comparable lifestyles, with the highest rates often occurring in aboriginal groups such as Pima Indians, Alaskan Natives, and Polynesian Islanders [3].

Because meaningful changes in the structure of the human genome occur over thousands of years, recent changes in lifestyle and not in genetics per se must have triggered the emergence of the complex diseases that are prevalent in many industrialized nations. This notion is supported by clinical trials, which show that with increased physical activity and improvements in dietary composition weight loss is achievable, cardiovascular risk profiles can be improved, and the rates at which type 2 diabetes occurs can be massively reduced [4, 5]; for most, adopting lifestyle behaviors similar to those of our ancestors reverses the progression of cardiometabolic disease. However, there is also considerable variability in the extent to which an individual responds to lifestyle intervention, with some being highly responsive and others maintaining similar risk profiles across the intervention period [6]. A fraction of this variability may be explained by gene-lifestyle interactions.
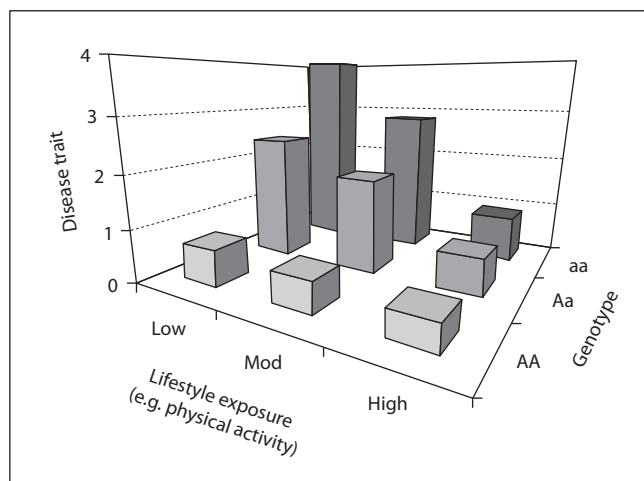
Fig. 1. A gene-lifestyle inter-action occurs when the effect of a lifestyle exposure on a disease trait differs in magnitude between genetic subgroups.

What Is a Gene-Lifestyle Interaction?

A gene-lifestyle interaction exists when the relationship between a lifestyle exposure (or treatment) and a disease trait differs in magnitude between genetic subgroups (e.g. genotypes at a specific genetic locus); a statistical test of interaction helps define the extent of these differences (fig. 1). For a gene-lifestyle interaction to truly occur (i.e. in a manner that is free from confounding or other sources of bias), the lifestyle exposure or treatment likely biologically affects the function or activity of the gene. The gene must also be functionally polymorphic, and facilitate the development of a relevant disease phenotype. Thus, although statistical tests of interaction rely on comparing numeric relationships, they aim to reflect underlying biologic interactions.

Historical Basis to the Concept of Gene-Lifestyle Interaction

In his 1962 paper in the *American Journal of Human Genetics* [7], James Neel presented the idea that, ' ... genes and combinations of genes which were at one time an asset may in the face of environmental change become a liability' (p. 359). This statement broadly defines the fundamental concept on which studies of gene-lifestyle interaction focus. In his paper, Neel set forth a new way of thinking about genetic and phenotypic variation which he termed the 'thrifty genotype' hypothesis. The hypothesis was an attempt to explain the high rates of type 2 diabetes seen in some aboriginal populations. Neel suggested that a 'thrifty' metabolism results in an altered postprandial insulin response which leads to a greater ability to store excess energy during periods of 'feast'. Those who possess this thrifty metabolism, Neel argued, would experience a survival advantage during periods of famine. Chakravarthy and Booth

[8] recently extended Neel's hypothesis to place emphasis on physical activity behavior. Their review suggests that current environments which require little in the way of physical activity for day to day survival are incompatible with the human genome which has remained largely unchanged in the last 10,000 years. This Late-Paleolithic genotype rewarded efficiency by regulating fuel utilization during periods of physical activity and rest. While some argue that the human genome has actually changed considerably during the past 10,000 years [9, 10], the underlying principle that a lag in human evolution has resulted in a situation where our genome is mismatched with the current environment remains plausible. This is especially true considering the rapid changes in the environment that occurred following the Industrial Revolution.

Despite widespread popularity, the thrifty genotype hypothesis has been the subject of criticism; although there does appear to be consensus that the recently observed increase in obesity is likely the result of gene-lifestyle interactions [7, 8, 11, 12]. A key critic of the thrifty genotype hypothesis is John Speakman [11–13]. Arguments presented by Speakman suggest that genes which favor excess energy storage have not experienced positive selective pressure in the past. Instead Speakman proposes that these genes have been subject to random genetic drift due to the absence of selective pressure [11, 13]. The criticism has been refined, but initial reasons for dismissing the thrifty genotype hypothesis focused on the association between leanness and mortality during famine [11]. Later criticism included an argument that it was mathematically improbable that the 'thrifty genotype' hypothesis could explain the current prevalence of obesity [13]. Speakman's hypothesis, labeled the 'drifty gene' or 'genetic drift' hypothesis, has also in turn received criticism [14]. A key point is that the 'drifty gene' hypothesis is primarily focused on mortality and does not adequately address the important role of fertility. It has also been pointed out that the arguments about the 'thrifty' or 'drifty' genotype hypotheses have not included real genetic data and are thus prone to error, bias and confounding. Moreover, populations tend to mobilize during famine. Migrating under such circumstances is physiologically stressful and impairs fertility. By consequence, populations that migrate during famine are likely exposed to selective pressures that may favor genetic lineages which are more physiologically robust to such conditions. However, the calculations on which Speakman bases his arguments do not take this possibility into account.

Why Do Gene-Lifestyle Interactions Occur?

According to our understanding of human evolution, genomic variants that interfere with reproduction should be extremely rare. However, many of the bona fide SNPs for cardiovascular and metabolic diseases are highly prevalent. Roughly half of all white people, for example, carry at least one copy of the rs9939609 FTO gene variant, which for every copy corresponds to ~1.5 kg greater body mass and, by consequence, a significantly increased risk of developing type 2 diabetes [15]. One explanation for

why SNPs associated with traits that interfere with reproduction are common is that historically they conveyed a survival advantage.

As described above, much of human evolution involved exposure to physically demanding environments where infection, thermic stress, periods of food deprivation, and a requirement to be physically active predominated. In this environmental context, an ability to lay down adipose reserves when food was plentiful would have been essential for survival. As illustrated by modern examples of lipodystrophy, anorexia nervosa, cachexia and athletic training, women with little or no adipose tissue are amenorrheic, infertile, and are prone to infection. Thus, given the important role adipose tissue plays in reproduction and survival, it is likely that the human genome is enriched with genes that promote fatness and inhibit leanness. Thus, obesity and its pathogenic consequences are natural responses to an environment that lacks physical stress.

How Might Information on Gene-Lifestyle Interactions Elucidate the Molecular Basis of Disease?

The effects of lifestyle exposures on disease risk are mediated by our genes. For example, specific nutrients (e.g. polyunsaturated fatty acids, sodium or zinc) and components of physical activity (e.g. shear stress, energy flux or hyperthermia) activate genes involved in cardiovascular or metabolic processes beneficial to health. By contrast, restriction of these exposures leads to genomic dysregulation. The identification of gene-lifestyle interactions sheds light on the genes involved in mediating and modifying the effects of specific lifestyle exposures (or treatments). Thus, bona fide examples of gene-lifestyle interactions may help identify specific drug, behavioral or nutritional targets for disease treatment or prevention.

How Might Information on Gene-Lifestyle Interactions Help Identify Those at High Risk of Disease?

It is becoming increasingly evident that the between-individual variability in the susceptibility to disease in a given environment is attributable in part to genetic variation. Figure 2 shows the interaction between the PPARG Pro12Ala genotype and dietary fat intake on change in visceral fat area during a clinical trial for diabetes prevention [16]; these data suggest that the effects of dietary fat composition on visceral fat accumulation are dependent on the genotype carried at the Pro12Ala locus. Those carrying the Ala12 allele in this study accumulated visceral adipose tissue at a greater rate than Pro12 homozygotes when the ratio of dietary polyunsaturated fats (PUFAs) was low; this contrasts the effect of the genotype on visceral fat accumulation when PUFA intake was high. This information might be used to identify people who are most

Fig. 2. Interaction between Pro12Ala genotypes and PUFA intake on 1-year change in visceral adiposity in the metformin treatment arm (n = 149). Data are means (95% CI). Adapted from Franks et al. [16].
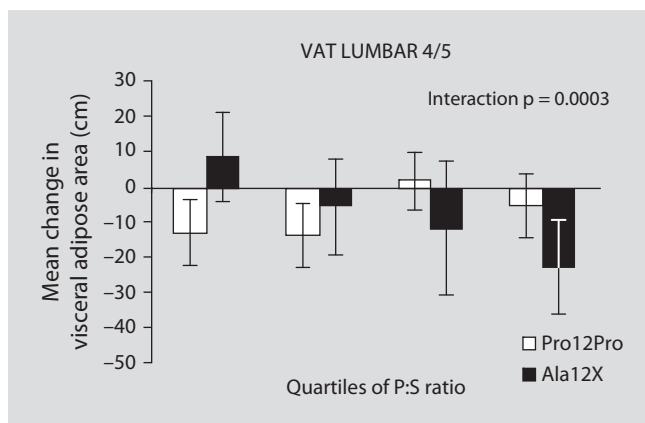
susceptible to become obese or develop cardiovascular disorders when eating diets high in saturated fats. These data also identify a genetic subgroup of individuals who appear to benefit most in terms of visceral fat reduction when consuming a diet high in PUFAs, raising the possibility of genome-guided prevention through nutritional intervention.

How Might Information on Gene-Lifestyle Interactions Be Used in the Treatment of Disease?

We have studied the interaction between the E23K variant at the KCNJ11 (potassium inwardly-rectifying channel, subfamily J, member 11) gene and metformin treatment on diabetes risk reduction in the DPP [17]. The overall reduction in diabetes risk with metformin treatment at 3.2 years was 32% in the DPP. However, as shown in figure 3, the reduction in risk was confined largely to people carrying the E23E genotype. No beneficial effect of metformin was observed in those carrying one or both copies of the K23 allele in our study. These data suggest that in carriers of the K23 allele at KCNJ11, metformin is an ineffective therapy. One might use these data to guide the choice of glucose-lowering medications. Because metformin shares some features in common with exercise (e.g. activation of the AMPK pathway and weight loss in the absence of muscle atrophy), understanding interactions between gene variants and metformin might shed light on the mechanism through which exercise interactions with our genes.

Figure 4 shows the interaction between the G62A variant at the G-protein-coupled receptor 10 gene and physical activity on blood pressure levels [18]. In people carrying the A allele (approximately 40% of the population), no relationship between physical activity and blood pressure was apparent. By contrast, in those carrying the GG genotype, a strong inverse relationship was observed. These data suggest that the

Fig. 3. Interaction between E23K genotypes and metformin treatment on diabetes incidence. Data are cumulative incidence. Adapted from Florez et al. [17].
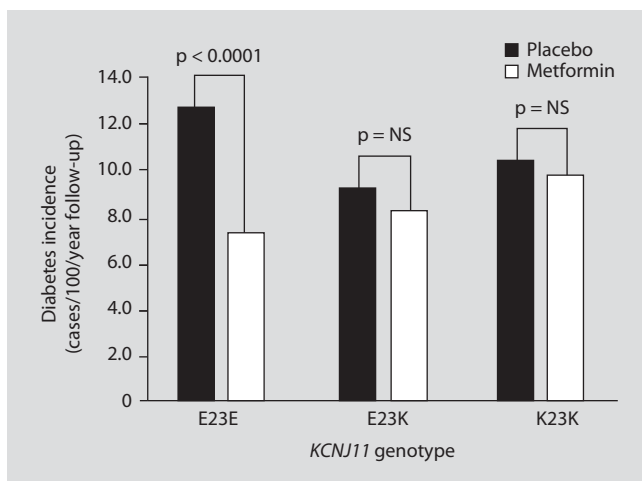
Fig. 4. Interaction between a polymorphism in the G-protein coupled receptor 10 gene and physical activity on blood pressure in 762 middle-aged adults from the UK. TEE/BMR = Total energy expenditure/basal metabolic rate. Reproduced from Franks et al. [18].
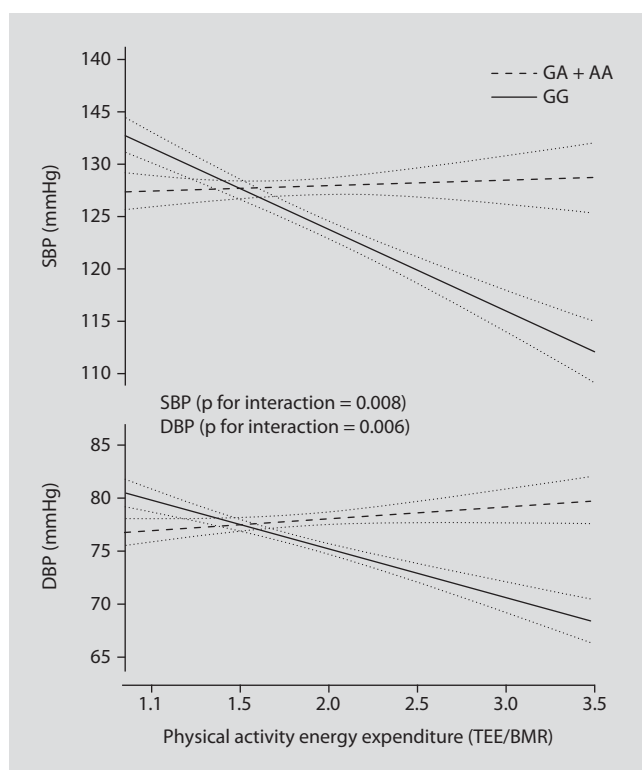
effects of physical activity on blood pressure may differ depending on genotype at this locus. Whilst physical activity has many other beneficial health effects and should rarely be discouraged, one might use this information on gene-lifestyle interaction to guide the choice of adjuvant antihypertensive therapies.

Overview of the Literature on Gene-Physical Activity Interactions on Human Obesity

The ability to undertake high-density genotyping in large population samples has facilitated a rapidly growing number of studies that report evidence of gene-exercise interactions on obesity-related traits (summarized in table 1). These studies include observational cohort, case-control, and intervention studies. Almost all studies published at the time of writing this chapter have focused on biological candidate genes or family-based linkage scans. Three studies in twins have also been reported [22–49]. Only a very small number of studies have tested variants identified through GWASs [22–51], which is probably owing to the recent emergence of these loci. These reports include a cohort study in which a variant at the FTO locus (rs9939609) was shown to interact with physical activity in around 5,000 middle-aged Danish adults [22]. In that study, the effect of the risk allele on obesity predisposition, which is now well established [15, 52], was clear in sedentary individuals, but was abolished in those who reported moderate to high levels of physical activity. In a subsequent cohort study set within around 700 members of the Amish community in Lancaster County, Pennsylvania, a second FTO gene variant (rs1861868), which is in weak linkage disequilibrium with the rs9939609 variant (r^2 ~0.17), also appeared to interact with physical activity (assessed using uniaxial accelerometry) [50]. Elsewhere, we examined whether the rs9939609 variant modifies the response to intensive lifestyle intervention in a randomized clinical trial of around 3,000 individuals at high risk of type 2 diabetes [53]. In that study, we did not find evidence to support an interaction between the rs9939609 variant and lifestyle intervention on changes in weight or BMI. However, we observed a modest interaction on 1-year change in visceral adipose volume determined through computed tomography. Although these studies might be considered confirmation of the initial Danish observation, it is important to stress that because the Amish study focused on a different FTO variant, and our study was set within the context of a clinical trial, the interaction was of borderline statistical significance and for a different obesity trait, appropriate confirmation studies are still required. To this end, we recently undertook a confirmation study focusing on the same variant and outcome studied in the Danish report [see 61]. Our study was undertaken in 16,000 Swedish adults living close to the region of Denmark in which the original report was set. The participants were of similar age and social status to the Danish participants. Nonetheless, we found no evidence to support the original study's finding that the rs9939609 variant and physical activity interact to influence obesity predisposition.

An interesting twist to the FTO story has been the recent discovery that persons carrying the rs9939609 risk alleles may be more physically active than those at lower genetic risk, despite being more obese. This observation was originally reported in Scottish children who underwent detailed assessments of energy intake and expenditure using doubly-labeled water [54], and confirmed in the Swedish cohort of 16,000 adults described above [55]. The explanation for this apparently paradoxical

Table 1. Overview of studies focusing on gene-physical activity interactions on obesity

Study	Study design	Description of cohort	PA assessment/ exposure
Observational studies			
Berentzen et al. [2004]	Prospective case-control study	• White males • Cases: n = 568; BMI 33.2 (2.3); age 32.0 (6.2) years • Controls: n = 717; BMI 21.5 (2.3); age 37.1 (8.6) years	Questionnaire
Franks PW et al. [2004]	Population-based cohort	Low physical activity level • Pro12Ala+ Ala/Ala Females: n = 26; BMI 25.5 (0.90); age 51.6 (1.79) years Males: n = 27; BMI 27.8 (0.64); age 54.6 (2.39) years • Pro12Pro Females: n = 114; BMI 26.9 (0.52); Age 53.9 (0.99) years Males: n = 86; BMI 27.0 (0.44); age 51.7 (1.26) years High physical activity level • Pro 12Ala+ Ala/Ala Females: n = 32; BMI 25.9 (0.68); age 48.8 (1.64) years Males: n = 22; BMI 26.5 (0.81); age 53.5 (2.63) years • Pro12Pro Females: n = 108; BMI 25.6 (0.42); age 51.7 (1.00) years Males: n = 91; BMI 26.9 (0.36); age 56.2 (1.01) years	4 days of free-living heart rate monitoring
Marti del Moral et al. [2002]	Case-control study	Cases: n = 159; BMI > 30 Controls: n = 154; BMI < 25; age 20–60 years	Questionnaire

Gene (variant)	Outcomes	Significant	Main results
UCP2 (3' UTR I/D) *UCP3* (−55 C/T)	Change in BMI, waist, and fat mass	No	Neither PA nor the gene variants were consistently associated with changes in BMI; there was no evidence for statistical interactions between the *UCP* variants and physical activity in relation to changes in any of the obesity measures
PPARG (Pro12Ala)	physical activity level, fasting insulin, polyunsaturated to saturated fatty acid ratio (P:S ratio)	Yes	In Pro allele homozygotes the combined associations of P:S ratio and PAL are additive; in contrast, in Ala allele carriers, PAL modified the association between P:S ratio and fasting insulin level in a multiplicative manner
ADRB3 (Trp64Arg)	BMI, M/S (metabolic equivalents divided with hours sitting down S) predict recreational energy expenditure	Yes	The risk of obesity associated with the *ADRB3* Trp64Arg polymorphism may be dependent physical activity levels; the effect (defined as odds ratio) of the polymorphism on obesity was 2.98 was apparent only among subjects with levels of recreational energy expenditure above the population median

Table 1. Continued

Study	Study design	Description of cohort	PA assessment/ exposure
Kilpeläinen et al. [2007]	Prospective cohort (4.1 years)	Three physical activity groups (n = 479) • Group 1: males/females, n = 53/n = 105; BMI 31.2 (4.1); age 56.1(7.0) years • Group 2: males/females, n = 50/n = 110; BMI 31.8 (5.1); age 53.9 (7.1) years • Group 3: males/ females, n = 56/n = 105; BMI 0.7(3.9); age 56.5(6.6) years	Questionnaire annually
Posion et al. [2001]	Cross-sectional study	African immigrants and African-American (n = 175) • CC or CT: n = 77 (88% females); BMI 29.9(6.5); age 42.4(7.5) years • TT: n = 98 (84.7% females); BMI 29.8 (4.9); age 42.8 (7.5) years	Self-reported physical activity (SRPA)
Moore et al. [2001]	Cross-sectional study	White postmenopausal non obese women (n = 63) • Sedentary: n = 19; BMI 23.7 (0.6); age 64 (1) years • Active: n = 20; BMI 25.2 (0.6); age 63 (1) years • Athletic: n = 24; BMI 21.7 (0.4); age 65 (1) years	VO_{2max}
Otabe et al. [2000]	Case-control study	• White obese females/males: n = 318/83; BMI 46.5 (7.5); age 44 (12) years • Control group females/males: n = 130/101; BMI 23(2.5); age 56 (14) years	Questionnaire
Ridderstråle et al. [2006]	Cross-sectional study	• Swedish men: n = 902; BMI 27.0 (4.6); age 48.0 (11.8) years • Swedish women: n = 899; BMI 26.8 (4.6); age 48.1 (11.9) years	Questionnaire

Gene (variant)	Outcomes	Significant	Main results
SLC2A2 (rs5393, rs5394, rs5404, s5400) *ABCC8* (rs2188966, rs1799859, rs3758947) *KCNJ11* (rs5219)	physical activity (METs), BMI, waist circumference, OGTT	Yes	Changes in moderate to vigorous physical activity during 4 years modified the effect of polymorphisms in *SLC2A* and *ABCC8* on glucose levels and the conversion from IGT to type 2 diabetes among individuals with IGT; the authors concluded that the interaction of physical activity with genes regulating insulin secretion indirectly suggests that physical activity not only improves insulin sensitivity but also preserve β-cell function.
GNB3 (CC, CT, TT)	physical activity, BMI	No	No statistically significant effect of the genotypes or interaction between genotype and the level of physical activity on BMI.
ADRB2 (Gln27Glu)	body fat %, fat mass, VO_{2max}, weight, BMI	Yes	Study concluded that the major Gln27 allele may be associated with elite athletic performance
UCP3 (−55 C>T)	physical activity index, BMI	Yes	Study suggested that the UCP3 variant may modify the association between physical activity and obesity
PPARGC1A (Gly482Ser)	BMI	Yes	Elderly men ≥50 years carrying 482Ser had an increased risk of obesity compared with subjects who were homozygous for the major allele; the risk was restricted to males with a low leisure-time physical activity level

Table 1. Continued

Study	Study design	Description of cohort	PA assessment/ exposure
Walston et al. [2003]	Cross-sectional study	White males and females (64%), African-American (30%), Hispanic (6%) • Trp64: n = 22; females 77.3%; BMI 29.4 (5.5); age 31.2 (8.6) years • Trp64Arg: n = 25; females 72.0%; BMI 28.2 (5.5); age 33.6 (8.5) years • Arg64: n = 15; females 86.7%; BMI 27.3(4.7); age 32.7 (10.9) years	Double-labeled water, questionnaire
Wootton et al. [2006]	Exercise intervention study	White males (n = 123) • AA: n = 77, BMI 23.13 (0.23); age 19.52 (0.29) years • AV: n = 39; BMI 23.08 (0.34); age 19.83 (0.44) years • VV: n = 7; BMI 23.07 (1.14); age 20.50 (0.24) years	10 weeks of intensive physical training
Ueno et al. [2006]	Cross-sectional study	Women (n = 97) • Glu12/Glu12: n = 78; BMI 33.7(0.3); age 33.2 (0.7) years • Glu12/Glu9: n = 13; BMI 33.7 (0.5); age 31.62 (1.7) years • Glu9/Glu9: n = 6; BMI 32.7 (1); age 32.17 (2.4) years	Responses to isometric handgrip exercise
Meirhaeghe et al. [1999]	Cross-sectional study	French men and women: n = 836; BMI ≥ 30; age 49.5 ± 8.1 years	Questionnaire
Andreasen et al. [2008]	Cross-sectional study	Danish men and women • TT: males/females, n = 969/1,008; BMI 25.9 (7.9), age 25.9 (7.9) years • TA: males/females, n = 1,423/1,360; BMI 26.2 (4.6); age 45.9 (8) years • AA: males/females, n = 461/501; BMI 27.0(4.9); age 46.5(8) years	Self-reported physical activity

Gene (variant)	Outcomes	Significant	Main results
ADRB3 (Trp64Arg)	resting metabolic rate (RMR), thermic effect of feeding (TEF), physical activity, RQ and postprandial RQ, BMI, body fat %, fat mass, fat-free mass, waist/hip ratio	Yes	RMR in Arg64 homozygotes was significantly lower than in Trp64 homozygotes; TEF was significantly higher in Arg64 homozygotes compared with Trp64 homozygotes; no differences were identified between genotypes in physical activity or in total energy expenditure
PLA2G7 (A379V)	BMI, blood pressure, adipose tissue mass, lean mass (full body MRI)	Yes	VV homozygotes experienced a significantly greater decrease in % adipose tissue and a significant greater increase in % lean mass compared with the AV and AA genotypes; the authors concluded that the association of this polymorphism with changes in body composition after training suggests a novel role for Lp-PLA2
ADRB2 (12Glu9deletion)	BMI, fat body mass, lean body mass, blood pressure, heart rate, lipids, glucose, insulin, leptin	Yes	*ADRB2* gene variant (Glu9/Glu9) appeared to be associated with reduced autonomic responsiveness by altering cardiac sympathetic and vagal function during sustained handgrip exercise in normotensive obese women
ADRB2 (Gln27Glu)	BMI , waist circumference, hip circumference, waist/hip ratio, physical activity	Yes	In inactive men, the risk of obesity associated with the Gln27Gln polymorphism was significantly increased while this risk was not significantly increased in active men
FTO (rs9939609)	BMI	Yes	The effect of the *FTO* rs9939609 A-allele on obesity was evident in sedentary individuals, but not in moderately-highly active individuals

Table 1. Continued

Study	Study design	Description of cohort	PA assessment/ exposure
Rampersaud et al. [2008]	Cross-sectional study	Amish men and women • AA: n = 166; 47.0% females; BMI 27.4 (4.8); age 43.4 (14.9) years • AG: n = 333; 49.6% females, BMI 26.5 (4.5); age 43.2 (14.1) years • GG: n = 203; 41.9% females; BMI 25.9 (4.1), age 44.6 (13.6) years	Uniaxial accelerometry
Boer et al. [1999]	Cross-sectional study	Dutch men and women • Carriers: males/females, n = 9/6 ; BMI 24.9 (3.3); age 39.8 (9.1) years • Noncarriers: males/females, n = 158/206; BMI 25.4 (3.8); age 41.0 (9.1) years	Questionnaire
de Luis Roman et al. [2006]	Exercise intervention study	Spanish women and men (n = 67); age 45.7(16.6) years overall • Lys656/Lys656: males/females, n = 10/26; BMI 33.8 (4.7) • Lys656/Asn656 and Asn656/ Asn656: males/females, n = 8/23; BMI 34.6 (5.2)	Aerobic exercise (60 min/3 times a week for 3 months)
Naito et al. [2007]	Cross-sectional study	Japanese men and women (n = 1234) Men: • A227: n = 95; BMI 23.1 (2.6); age 39.0 (9.6) years • V227: n = 894: BMI 22.9 (2.8); age 38.2 (9.1) years Women: • A227: n = 25; BMI 21.0 (2.8); age 34.8 (9.9) years • V227: n = 220; BMI 21.0 (2.9); age 33.1 (8.8) years	Questionnaire

Gene (variant)	Outcomes	Significant	Main results
FTO (rs1861868)	BMI	Yes	The effect of the *FTO* rs1861868 minor allele on obesity was evident in sedentary individuals, but not in moderately-highly active individuals
LPL (D9N)	BMI, serum lipids	Yes	The association between the *LPL* gene polymorphism and lipid levels was evident in inactive individuals, but not in active individuals
LEPR (Lys656Asn)	BMI, weight, fat-free mass kg, fat mass kg, waist circumference, waist/hip ratio, blood pressure, blood analysis; adipocytokines	Yes	Carriers of the minor allele at the *LEPR* gene had significantly greater responses to the exercise intervention than noncarriers with respect to decreases in BMI, weight and LDL cholesterol levels; not significant changes in leptin levels
PPARA (V227A)	HDL-C, LDL-C, total cholesterol, physical activity	Yes	The authors observed a significant interaction between the *PPARA* V227A polymorphism and physical exercise on HDL-C levels, such that exercise and HDL-C levels were positively associated in V allele homozygotes, but no effect was evident in minor allele carriers

Table 1. Continued

Study	Study design	Description of cohort	PA assessment/ exposure
Intervention studies			
Chagnon et al. [2001]	Exercise intervention study (HERITAGE study	99 families (192 parents, 330 offspring) Parents (n = 192) • Females: n = 95; BMI 27.6, age 52.0 years • Males: n = 99; BMI 28.4; age 53.5 years Offspring (n = 330) • Females: n = 172; BMI 23.7; age 25.4 years; • Males: n = 164; BMI 25.7; age 25.2 years	20-week aerobic exercise training
Kahara et al. [2002]	Exercise intervention study	Japanese males, n = 106; BMI 23.3 (2.8); age 45.4 (11.5) years • Trp/Trp: n = 74 • Trp/Arg: n = 25 • Arg/Arg: n = 7 • AA: n = 34 • AG: n = 45 • GG: n = 27	exercise program (2–3 days/week for 3 months)
Thompson et al. [2006]	Clinical trial, exercise intervention study	n = 110; females/males, n = 53/57; BMI 27.6 (5.0); age 38.9 (10.9) years	6 months training program
Yoshida et al. [1995]	Exercise intervention study	Japanese women (n = 88) Age for total: 54.5 (11.8) years • Trp64Arg homo: n = 5; BMI 34.4 (2.4) • Trp64Arg hetero: n = 30; BMI 33.3 (3.6) • Normal homo: n = 53; BMI 32.7 (3.5)	3 months exercise and low-calorie diet
Tworoger et al. [2004]	Exercise intervention randomized study	White women 87%, non-White women 12% and unknown women 1% • Exercise group: n = 87; BMI 30.5 (4.1); age 60.7 (6.7) years • Control group: n = 84; BMI 30.5 (3.7); age 60.7 (6.7) years	1 year exercise program

Gene (variant)	Outcomes	Significant	Main results
Genome-wide linkage scan	BMI, % fat, fat mass, fat-free mass, skinfold thickness, plasma leptin levels	Yes	Observed significant linkages for a body composition phenotype in response to a 20-week exercise training period and markers within S100A1 and IGF1 genes
ADRB3 (Trp64Arg) *UCP1* (AA, AG, GG)	body weight, blood pressure, total cholesterol, triglyceride, HDL-c, leptin, fasting glucose, fructosamine, insulin	Yes	ADRB3 polymorphisms were found to be associated with exercise-mediated improvements in several of the traits examined; The UCP1 polymorphism also appeared to modify the effects of exercise on glucose tolerance and possibly leptin resistance
ACE (I/D) *APOE* (2/3, 3/3, 4/3)	$VO_{2\,max}$, BMI, body fat %, serum lipids, % maximal heart rate, adherence	Yes	Authors conclude that the presence or absence of the ACE insertion allele may affect adherence to exercise training
ADRB3 (Trp64Arg, Try64Arg)	waist/hip ratio, BMI, body weight loss, adjusted resting metabolic rate, visceral/ subcutaneous fat ratio, TSH and free thyroxine	Yes	In obese individuals, the Trp64Arg polymorphism was associated with lower resting metabolic rate; that group also lost less weight during 3 months lifestyle intervention, although this did not reach a level of statistical significance
CYP19A1 COMT (Val108/158Met)	BMI, total body fat, % body fat, subcutaneous fat (CT), intra-abdominal fat (CT)	Yes	Exercisers with two vs. no CYP19 11-repeat alleles had a larger decrease in total fat and percentage body fat; exercisers with the COMT Met/Met vs. Val/Val genotype had a smaller decrease in percentage body fat; among exercisers, women with the COMT Val/Val genotype and at least one copy of the CYP 19 11-repeat allele vs. those with neither genotype had a significantly greater decrease in BMI, total fat and percentage body fat

Table 1. Continued

Study	Study design	Description of cohort	PA assessment/ exposure
Wootton et al. [2006]	Exercise intervention study	White males (n = 123) • AA: n = 77; BMI 23.13 (0.23); age 19.52 (0.29) years • AV: n = 39; BMI 23.08 (0.34); age 19.83 (0.44) years • VV: n = 7; BMI 23.07 (1.14); age 20.50 (0.24) years	10 weeks of intensive physical training
Thamer et al. [2008]	Exercise intervention study	Males/females, n = 61/95; BMI 29.0 (19.4, 43.5); age 47 (19,68) years	9 months exercise and diet program = LI
Rice et al. [1999]	Exercise intervention study	White families • Fathers: n = 83; BMI 28.2 (4.5); age 53.5 (5.1) years • Mothers: n = 80; BMI 27.2 (5.0); age 52.1 (5.0) years • Sons: n = 126; BMI 25.4 (4.4); age 25.4 (6.1) years • Daughters: n = 151; BMI 23.6 (4.5); age 25.6 (6.5) years	20 weeks of exercise program
de Luis Roman et al. [2006]	Exercise intervention study	Spanish women and men (n = 67); age 45.7 (16.6) years overall • Lys656/Lys656: males/females, n = 10/26; BMI 33.8 (4.7) • Lys656/Asn656 and Asn656/Asn656: males/females, n = 8/23; BMI 34.6 (5.2)	Aerobic exercise (60 min/3 times a week for 3 months)
Hereditary and twin studies			
Samaras et al. [1999]	Cross-sectional study	White female twins (n = 970); BMI 24.4 (16.4–44.0); age 55.5 (39.0–70.0) years	Questionnaire

Gene (variant)	Outcomes	Significant	Main results
PLA2G7 (A379V)	BMI, blood pressure, adipose tissue mass, lean mass (full body MRI)	Yes	VV homozygotes experienced a significantly greater decrease in % adipose tissue and a significant greater increase in % lean mass compared with the AV and AA genotypes; the authors concluded that the association of this polymorphism with changes in body composition after training suggests a novel role for Lp-PLA2
PPARD (rs1053049, rs6902123, rs2267668)	body weight, BMI, visceral adipose tissue, nonvisceral adipose tissue, hepatic lipids, muscle volume, fasting glucose, OGTT, insulin sensitivity, habitual physical activity	Yes	Carriers of the minor alleles displayed reduced responses to LI in reduction of adipose tissue mass and hepatic lipids as well as LI-induced increase in lower body muscle mass
Genome-wide linkage scan	fat mass , abdominal visceral fat (AVF)	Yes	Study found little evidence of multifactorial heritability for either AVF response or FM response to exercise intervention; the authors concluded that the exercise training responsiveness appears to be primarily due to major genes (a recessive locus for AVF response and dominant locus for FM response
LEPR (Lys656Asn)	BMI, weight, fat-free mass kg, fat mass kg, waist circumference, waist/hip ratio, blood pressure, blood analysis; adipocytokines	Yes	Carriers of the minor allele at the LEPR gene had significantly greater responses to the exercise intervention than noncarriers with respect to decreases in BMI, weight and LDL cholesterol levels; not significant changes in leptin levels
Twin-based heritability study	total body fat %, central abdominal fat	No	No evidence of an interaction between gene variant and physical activity on total body or central abdominal fat mass

Table 1. Continued

Study	Study design	Description of cohort	PA assessment/ exposure
Ortega-Alonso et al. [2008]	Cross-sectional study	Finnish twins (n = 394) • Monozygotic: n = 184; BMI 27.92 (4.85); age 67.90 (3.55) years • Dizygotic: n = 210; BMI 27.75 (4.70); age 68.78 (3.08) years	Walking speed test, walking endurance test
Karnehed et al. [2006]	Prospective cohort (4 years) Swedes male twins	Swedish male twins • Monozygotic: n = 574; BMI (at follow-up) 23.5 (0.15); age 25.6 (0.11) years • Dizygotic: n = 378; BMI (at follow-up) 4.0 (0.19); age 25.8 (0.14) years	Questionnaire
Feitosa et al. [2000]	Cross-sectional study	Indian families • Parents: n = 669; BMI 22.082 (3.995); age 42.892 (11.013) years • Offspring: n = 772; BMI 17.618 (3.188); age 17.487 (7.944) years Sex ratio not specified	Interview

LI = Low intensity; OGTT = oral glucose tolerance test; RQ = respiratory quotient; PAL = physical activity level.

observation is that the propensity for FTO risk allele carriers to be more physically active is offset by their even greater tendency to overeat [54].

As shown in table 1 the vast majority of published studies on gene × lifestyle interactions have been undertaken in relatively small sample sizes with subjective methods used to assess physical activity (usually questionnaires). Whilst these studies have formed the bedrock of this area of research, the reality is that many are likely to be grossly underpowered for the detection of interactions. By consequence, many of the published reports of gene-lifestyle interactions are likely to be susceptible to type 2 error (i.e. false positivity). With this sobering thought in mind, it is clear that, as others have accepted in the field of human genetics, it will be necessary to increase sample sizes and improve the precision with which lifestyle exposures are assessed. Human genetics researchers studying complex disease traits have recognized the need for larger sample sizes and through international consortiums have comprised

Gene (variant)	Outcomes	Significant	Main results
Twin-based heritability study	body fat % BiA, BMI, walking speed, walking distance in 6 min	Yes	The authors observed a negative association between body fat and walking speed that was largely due to cross-trait genetic effects; they also observed a negative correlation between body fat and walking endurance; the authors concluded that among community-living older women, the inverse associations between adiposity and mobility are mostly due to the effect of shared genes
Twin-based heritability study	Occupational and leisure time, waist circumference, BMI	Yes	The authors reported evidence to suggest that the heritability of obesity is modified by physical activity levels
Family-based heritability study	BMI, energy expenditure of activity level, energy intake	Yes	The cross trait resemblance between the two measures (BMI in parents with BMI as predicted from the energy variables in the offspring) was significant for all biological relatives, indicating the presence of shared genetic or environmental determinants which accounted for 58% of the trait variation; cross-trait spouse correlation indicated a part of the resemblance was due to nongenetic effects

cohort collections involving many thousands of participants. Unfortunately, owing to the statistical constraints imposed when testing hypotheses of gene-lifestyle interaction, such studies will need to be an order of magnitude larger than those focusing only on genetic effects to attain comparable statistical power [56]. To this end, several international consortiums have been formed to study gene-lifestyle interactions on complex disease traits, the results of which should emerge in the near future.

Conclusions

Studies of gene-lifestyle interaction help elucidate the molecular mechanisms through which physical activity favorably impacts health. Such studies also shed light on the ways in which exercise interventions might one day be tailored to the patient's

genotype, thus improving the effectiveness of the intervention. However, these prospects are contingent on the discovery, confirmation, and refinement of genetic loci that interact with lifestyle behaviors to influence disease risk and progression. Ideally, this evidence should include studies from appropriately conducted randomized controlled trials. Despite a burgeoning literature on gene-lifestyle interactions, there are few (if any) convincing examples of genetic loci that act in this way. Indeed, as we have previously argued, no appropriately designed intervention studies have been reported that test whether variability in phenotypic responses to exercise intervention is attributable to genetic variation [57]. Although studies such as HERITAGE clearly illustrate that the phenotypic response to exercise varies greatly from one person to the next and that this is related to inherited factors [6], nongenetic factors are also inherited [59]. Moreover, even though studies such as HERITAGE [6] and DREW [58] diligently administered the exercise intervention, physical activity (and other behaviors) outside the training sessions was not tightly controlled; these factors rather than genetic variation may explain why people responded differently to the same exercise regime [60]. Having said this, given what we understand of human evolution, it remains highly intuitive that genetic variation influences exercise response. Thus, to move from the stage of speculating about the combined role of genetics and exercise for health promotion and disease prevention to one where this becomes reality will require a paradigm shift. This means that the standards which have become commonplace elsewhere in human genetics research, where reports of genetic associations with disease traits undergo extensive replication efforts before being accepted as real, will need to be adopted by those of us focusing on gene-lifestyle interactions if our work is to meaningfully impact human health.

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Genetic Risk Factors for Musculoskeletal Soft Tissue Injuries

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Abstract

Acute and overuse musculoskeletal soft tissues injuries are common as a result of participating in specific physical or workplace activities. Multiple risk factors, including genetic factors, are implicated in the aetiology of these injuries. Common musculoskeletal soft tissue injuries for which a genetic contribution has been proposed include the Achilles tendon in the heel, the rotator cuff tendons in the shoulder and the cruciate ligaments in the knee. Recent developments in the identification of genetic risk factors for tendon and ligament injuries will be reviewed. Sequence variants within genes that encode for several tendon and/or ligament extracellular matrix proteins have been shown to be associated with specific musculoskeletal soft tissues injuries. Variants within the *TNC, COL5A1* and *MMP3* genes co-segregate with chronic Achilles tendinopathy. The variant within the *TNC* gene also appears to co-segregate with Achilles tendon ruptures, while sequence variants within the *COL1A1* and *COL5A1* genes have been shown to be associated with cruciate ligament ruptures and/or shoulder dislocations. Whether these variants are directly involved in the development of these musculoskeletal soft tissue abnormalities or in strong linkage disequilibrium with actual disease-causing loci remains to be established. We proposed that genetic risk factors will in the future be included in multifactorial models developed to understand the molecular mechanisms that cause musculoskeletal soft tissue injuries or related pathology. Clinicians could eventually use these models to develop personalised training programmes to reduce the risk of injury as well as to develop treatment and rehabilitation regimens for the injured individual.

Tendons and ligaments are common structures that can be injured as a result of participating in physical activity or specific activities in the workplace [1]. Collectively, these injuries, together with injuries to skeletal muscle, are referred to as musculoskeletal soft tissue injuries and can be broadly classified as either acute or overuse injuries [2]. Acute injuries result from a specific macrotraumatic event, while overuse injuries result from cumulative microscope damage to tissues caused over time by

repetitive microtraumatic events. Although initially asymptomatic, the increased volume of tissue damage eventually becomes symptomatic [2].

Common musculoskeletal soft tissue injuries which will be covered in this review include the Achilles tendon in the heel, the rotator cuff tendons in the shoulder and the cruciate ligaments in the knee. Rupture of the Achilles tendon and anterior cruciate ligament (ACL) are examples of acute injuries, while Achilles tendinopathy (also historically referred to as Achilles tendonitis) is an example of an overuse injury. As much as 50% of all sporting injuries involve tendons of which up to a fifth can comprise Achilles tendon injuries, while the lifetime prevalence of Achilles tendon injuries has been reported to be as high as 11% [3–5]. Although the incidence of ACL injuries is low in the general population [6, 7], regular participation in sports, particularly those which involve a sudden deceleration or change in direction, may place an individual at up to ten times greater risk of ACL rupture [8]. ACL ruptures are considered one of the most severe injuries sustained in a sporting population [9].

Although the genetic basis of tendon and ligament injuries has been reviewed [10, 11], this chapter will focus on the more recent developments in the field.

Tendon and Ligament Structure

Tendons and ligaments are collagenous structures which have a similar composition and hierarchical structure [12–14]. Approximately two thirds of these tissues consist of water. The rest is made up of tightly packed parallel bundles consisting predominately of type I collagen fibrils (60–80% of the dry mass of tendons). The fibrils aggregate into larger units of fibres and fascicles which finally produce the tendon or ligament [14].

Other collagen types, such as types III–VI, XII and XIV, are also structural components of the fibrils and surrounding extracellular matrix (ECM; fig. 1) [13, 15–18]. Various proteoglycans, such as decorin, lumican and versican, and glycoproteins, such as elastin, tenascin C and COMP, are also important components of tendons and/or ligaments [13–15, 19, 20]. The quantity of these other collagens, proteoglycans and glycoproteins within these tissues are however significantly less than type I collagen. Regions within tendons which have to also resist, in addition to the usual tensile forces, compressive and shear forces, also contain fibrocartilaginous-specific proteins such as types II, and IX–XI collagen, as well as the proteoglycans, biglycan and aggrecan [17, 18]. The genes that encode for these proteins are located throughout the human genome [10]. Changes in the collagen, proteoglycan and/or glycoprotein content and composition, as well the expression of many of the genes that encode for these proteins have been shown to be altered in tendinopathy [16, 18, 21, 22] and ligament injuries [23].
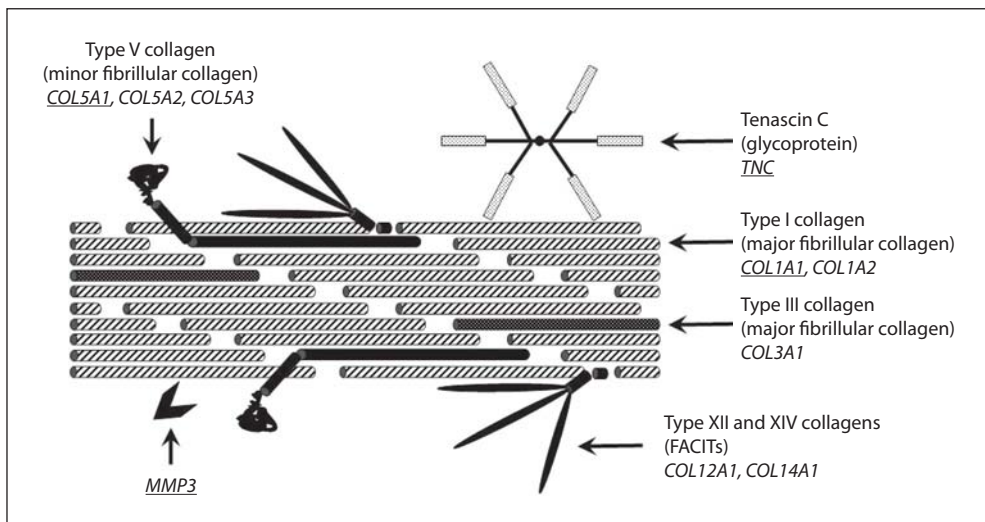
Fig. 1. A schematic diagram of the basic structural unit of tendons and ligaments, the collagen fibril. The fibril consists predominately of type I collagen. Type I collagen is a heterotrimer consisting of two α1(I) and one α2(I) chains, which are encoded for by the *COL1A1* (human chromosomal location 17q) and *COL1A2* (7q) genes, respectively. The fibril also contains trace amounts of types III and V collagen. Type III collagen is a homotrimer consisting of three α1(III) chains encoded for by the *COL3A1* (2q) gene. The major isoform of type V collagen is a heterotrimer consisting of two α1(V) and one α2(V) chains, which are encoded for by the *COL5A1* (9q) and *COL5A2* (2q) genes, respectively. Some isoforms contain an α3(V) chain encoded for by the *COL5A3* gene (19q). Types I and III collagen are members of the major fibrillular collagen sub-family, while type V collagen is a member of the minor fibrillular collagen sub-family. Types XII and XIV collagen are associated with the surface of the fibril and belong to the sub-family of fibril-associated collagens with interrupted triple helices (FACITs). Both proteins are homotrimers which are encoded for by the *COL12A1* (6q) and *COL14A1* (8q) genes. The hexameric glycoprotein, tenascin C and MMP3 are also shown. Tenascin C and MMP3 are encoded for by the *TNC* (9q) and *MMP3* (11q) genes, respectively. Other collagen types, proteoglycans and glycoproteins which are associated with or structural components of the fibril are not shown. The genes that have been shown to be associated with any musculoskeletal soft tissue injuries are underlined. The proteins are not necessarily drawn to scale.

Mechanism of Injury

The exact mechanisms responsible for tendon and ligament injuries are poorly understood [17, 24]. Since multiple risk factors are implicated in the aetiology of these injuries, these injuries are considered multifactorial disorders [17, 24]. The risk factors are divided into those which influence the athlete from without (extrinsic) and those from within (intrinsic) [2]. It has been proposed that the intrinsic risk factors predispose the athletes to a specific injury. Once predisposed, susceptibility to injury is determined by exposure to extrinsic risk factors. It is important to note that these intrinsic and extrinsic risk factors do not cause or necessarily result in an injury. The occurrence of a specific inciting event eventually results in an injury (fig. 2). The
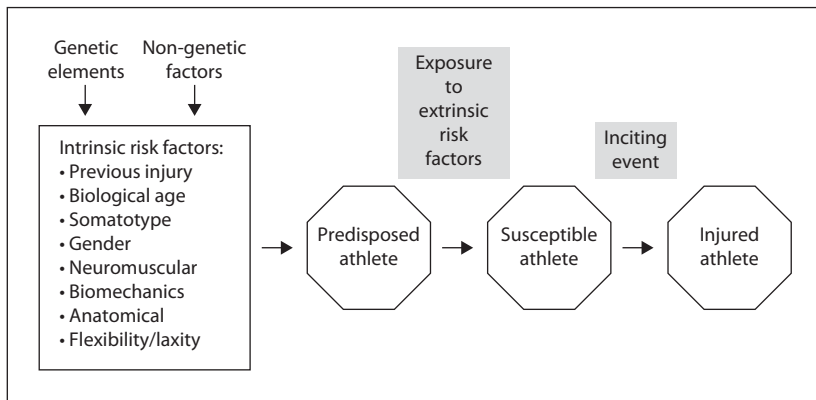
Fig. 2. A schematic diagram illustrating the complex relationship between intrinsic and extrinsic risk factors, as well as the role of the inciting event in the aetiology of soft tissue injuries [2, 25, 26]. Many intrinsic risk factors are, together with several non-genetic factors, determined to a lesser or greater extent, by genetic elements and individually could also be considered as multifactorial phenotypes.

relationship between intrinsic and extrinsic risk factors, as well as the role of the inciting event in the aetiology of sports injuries has been extensively reviewed [2, 25, 26].

Genetic factors have been suggested as intrinsic risk factors for Achilles tendon [27–30], rotator cuff tendon [31], shoulder dislocations [32] and ACL [32–36] acute and/or chronic injuries. Although the sequences of the 3.2 billion bases of human DNA are over 99.9% identical, the sequence variations (polymorphisms) in human DNA contribute to the visible and measurable biological variation observed in individuals. In fact, many of the common intrinsic risk factors implicated in injuries such as previous injury, somatotype, neuromuscular characteristics, biomechanical features, anatomical features and flexibility/laxity are, together with several other non-genetic factors, to a lesser or greater extent, determined by genetic factors [37]. Many of the intrinsic risk factors could therefore also in their own right be considered as multifactorial phenotypes. We hypothesize that polymorphisms within specific genes are partially responsible for the observed inter-individual variation in susceptibility to musculoskeletal soft tissue injuries.

Type I Collagen Genes and Musculoskeletal Soft Tissue Injuries

As previously mentioned, type I collagen is the major protein component of tendons and ligaments [14]. The protein is a heterotrimer consisting of two α1(I) and one α2(I) chains, which are encoded for by the *COL1A1* and *COL1A2* genes, respectively (fig. 1) [38]. Mutations within the *COL1A1* gene have been shown to cause connective tissue disorders such as osteogenesis imperfecta, and Ehlers-Danlos syndrome
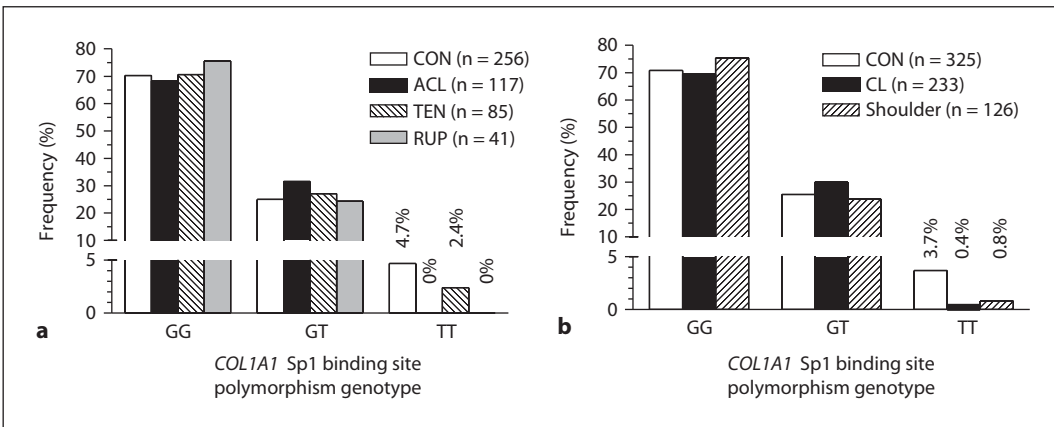
Fig. 3. The relative genotype frequencies of the functional Sp1 binding site polymorphism within intron 1 of the *COL1A1* gene in the South African control (CON), ACL rupture, chronic Achilles tendinopathy (TEN) and Achilles rupture (RUP) groups (**a**), as well as the Swedish CON and cruciate ligament (CL) rupture and shoulder dislocation (Shoulder) groups (**b**). The data from both South African Caucasian control populations, which consisted of unrelated individuals, were combined and presented [35, 46]. **a** CON versus ACL, OR = 12.0, 95% CI 0.7–204.8, p = 0.022; CON versus TEN, OR = 2.0, 95% CI 0.4–9.3, p = 0.531; CON versus RUP, OR = 4.2, 95% CI 0.2–73.1, p = 0.383. **b** CON versus CL, OR = 8.9, 95% CI 1.1–68.9, p = 0.010; CON versus Shoulder, OR = 4.8, 95% CI 0.6–37.3, p = 0.123.

[39]. Various single nucleotide polymorphisms within *COL1A1* have also been shown to be associated with several complex connective tissue disorders [40–44]. The G to T substitution in an intronic Sp1 binding site (rs1800012), resulting in increased affinity for the transcription factor Sp1, and increased gene expression, has been one of the most extensively investigated polymorphism within *COL1A1* [45]. With respect to musculoskeletal soft tissue injuries, the association of this polymorphism with cruciate ligament ruptures [32, 35], shoulder dislocation [32], Achilles tendon ruptures [46] and Achilles tendinopathy [46] has been investigated (fig. 3).

The rare TT genotype was found to be absent in South African Caucasian subjects with ACL (n = 117) [35] or Achilles tendon (n = 41) [46] ruptures (fig. 3a). In a Swedish study, Khoschnau et al. [32] only identified one subject each with a TT genotype with cruciate ligament ruptures (0.4%, n = 233) or shoulder dislocations (0.8%, n = 126; fig. 3b). Two subjects with the rare TT genotype were however identified in the subjects with chronic Achilles tendinopathy (2.4%, n = 85) [46] (fig. 3a). Similar TT genotype frequencies were reported in the control subjects in both the South African (4.7%, n = 256) and Swedish (3.7%, n = 325) studies, which is in agreement with the genotype frequencies reported in larger control populations [45, 47]. When combined, the rare TT genotype was significantly underrepresented in the subjects with either ligament or tendon ruptures (0.4%, n = 2 of 517) when compared with the control subjects (4.1%, n = 24 of 581, p < 0.0001) [48]. Although the rare TT genotype appears to have a protective role against acute soft tissue ruptures, larger sample sizes

should be evaluated in the future to further investigate the effect of this genotype on various ligament, tendon and other soft tissue ruptures.

It is also interesting to note that, in the South African study, subjects with an ACL rupture were more than four times as likely to have a blood relative with a history of a ligament injury when compared with the control subjects [35]. This supports previous research that reported a congenital component [33] and familial genetic predisposition [34] to ACL injuries. It is however possible that the family members of the ACL subjects in the South African study have a higher exposure to non-genetic risk factors, such as participation in sports which involve a sudden change in direction or deceleration, compared with the family members of the control subjects.

The possible association of any other polymorphisms within the 15.8 kb *COL1A1* gene, which consists of 51 exons, or any polymorphisms within the 52 exons and 51 introns of the 36.3 kb *COL1A2* gene with soft tissue injuries has to date not been investigated.

Soft Tissue Injuries and Chromosome 9

It is highly unlikely that a single gene or a single variant within one gene exclusively predisposes athletes to musculoskeletal soft tissue injuries. These injuries, like other multifactorial disorders and phenotypes [11, 49, 50], are more likely polygenic in nature. However, which of the other 20,000–25,000 protein-coding genes located throughout the human genome are associated with these injuries? Although beyond the scope of this review, it is also important to mention that non-protein-coding genes could also be considered as possible genetic risk factors for these injuries.

Technologies have been developed and are available to screen the whole human genome in a single experiment for identifying the genetic differences between cohorts [51]. Although one advantage of this technology is obtaining large amounts of genetic information, it requires large sample sizes, is expensive and is often not hypothesis driven. Another approach for identifying genes associated with musculoskeletal soft tissue injuries is to select and test specific candidate genes based on their known biological function [10, 11]. Although this approach also has certain limitations [52, 53], it requires smaller sample sizes, is hypothesis driven and, as reviewed by September et al. [10, 11], it has successfully been used to identify and test candidate genes for Achilles tendon injuries based on their biological function and chromosomal location.

Some investigators have reported an association of the ABO blood group with Achilles tendon injury [10]. Furthermore, the ABO blood group is determined by a single gene located on the end of the long arm of chromosome 9 (9q34). Therefore, based on their biological function and chromosomal location the *COL5A1* and *TNC* genes were selected as candidate genes for Achilles tendon injuries [29, 30]. The *COL5A1* and *TNC* genes encode for the α1 chain of type V collagen and tenascin C,

respectively [10, 11]. Type V collagen molecules intercalate into the tendon and ligament fibrils where they are believed, together with other proteins, to play an important role in regulating fibrillogenesis and modulating fibril growth [15, 17, 54] (fig. 1). Tenascin-C, on the other hand, is localised predominantly in regions responsible for transmitting high levels of mechanical force within tendons and ligaments [55]. The expression of the gene is regulated by mechanical loading [55–57].

It was found that a GT dinucleotide repeat polymorphism within intron 17 of the *TNC* gene is associated with both chronic Achilles tendinopathy and Achilles tendon rupture. Variants containing 12 and 14 GT repeats were overrepresented in individuals with either injury, while variants containing 13 and 17 repeats were underrepresented. In addition to the *TNC* gene, a *Bst*UI restriction fragment length polymorphism (RFLP) (rs12722) within the 3′-untranslated region of the *COL5A1* gene was also associated with chronic Achilles tendinopathy in South African [30] and Australian populations [58]. In both populations, the CC genotype was underrepresented by greater than 2-fold in the tendinopathy patients. Although only investigated in a small cohort, the *COL5A1 Bst*UI RFLP does not appear to be associated with Achilles tendon ruptures [30]. More recently, Posthumus et al. [36] have shown that the CC genotype of this polymorphism was also significantly underrepresented in female, but not male, athletes with ACL ruptures. The possible role(s) of type V collagen and/or tenascin-C in the development of soft tissue injuries needs to be investigated.

Other Matrix Genes

Since associations of the *COL5A1* and *TNC* genes with Achilles tendon and/or ACL injuries have already been described, any gene that encodes for other ECM proteins involved in similar biological processes as type V collagen and tenascin-C within these tissues would also be an ideal candidate gene for musculoskeletal soft tissue injuries. For example, proteins, such as decorin [59], lumican [60], fibromodulin [60], thrombospondin 2 [61] and types XII and XIV collagen [62–64] regulate fibrillogenesis and, therefore, their genes could also be investigated as genetic markers for these injuries.

Besides tenascin-C, the homotrimeric types XII and XIV collagen, which are also expressed in both tendons and ligaments, are regulated by mechanical loading [55, 65] (fig. 1). In particular, the small XIIB-1 isoform of type XII collagen is predominately expressed in tendons and ligaments [66]. These molecules belong to the subfamily of fibril-associated collagens with interrupted triple helices (FACIT) [67–69] which are believed to form interfibrillar connections and mediate fibril interaction with other extracellular and cell surface molecules [70–73] (fig. 1). Sequence variants within the *COL12A1* and *COL14A1* genes, which encode for types XII and XIV collagen, respectively, were individually not associated with Achilles tendinopathy [74].

Although not significant, the absence of both the rare CC and GG genotypes of the *COL12A1 Bsr*I and *Alu*I RFLPs, respectively, in the Achilles tendon rupture subjects warrants further research [74]. The AA genotype of the *COL12A1 Alu*I RFLP has recently been shown to be significantly overrepresented in female, but not male, subjects with ACL ruptures [see 99].

Matrix Metalloproteinases and ADAMTSs

The integrated nature of biological systems implies that multiple genes which encode for other structural components of tendons and ligaments or biological processes, such as adaptation, healing and remodelling, are probably also associated with these injuries. Besides the genes which encode for the ECM proteins already discussed, the genes that encode for any protein that regulates tendon and ligament biology,such as matrix metalloproteinases (MMPs), ADAMTSs (a disintegrin like and metallo-protease-thrombospondin with type 1 motif), tissue inhibitor of metalloproteinases, growth factors, cytokines and interleukins involved in musculoskeletal soft tissue biology are also ideal candidates [11]. Although all these families of proteins are important, only the MMPs and ADAMPTs will be further discussed in this review.

The MMPs are a family of at least 20 distinct endopeptidases that catalyse a plethora of ECM- and non-ECM-associated substrates [75]. MMPs have important roles in a variety of biological processes including wound healing [76], angiogenesis [77] and the regulation of various types of tissue turnover [78]. Although the genes that encode for the MMPs have been mapped throughout the human genome, many form a cluster on chromosome 11 (www.ncbi.nlm.nih.gov).

There is ample evidence to show that the expression of several *MMP*s is altered in damaged tendons [79, 80]. Furthermore, it has recently been demonstrated that expression of *MMP3* and *MMP13* is influenced by changes in mechanical loading in the Achilles and supraspinatus tendons [81]. In addition, serum levels of both MMP2 and MMP7 appear to be raised in individuals with a history of Achilles tendon rupture [82], and synovial fluid collected from the knees of individuals with ACL injuries is reported to have elevated levels of the MMP3 protein [83].

Alteration of *MMP* transcription in relation to musculoskeletal soft tissue injury has generated interest in *MMP* genes per se in Achilles-related pathology in human populations [11, 84]. Indeed, variation within *MMP* genes is associated with a variety of diseases including lung and oesophageal cancer [85, 86], inflammatory bowel disease [87] and invertebral disc degeneration [88]. In terms of musculoskeletal soft tissue injuries, we have recently shown that three common variants within the *MMP3* gene (rs679620, rs591058 and rs650108) are associated with Achilles tendinopathy in a South African Caucasian population [89]. Individuals that are homozygous for the risk alleles at the above loci are at least twice more likely to develop Achilles tendinopathy than individuals that do not have the risk genotypes. Interestingly, the study

also found that the G-allele of rs679620 interacted with the T-allele of rs12722 (within the *COL5A1* gene) to further increase risk of tendinopathy [89]. Future work should attempt to establish whether *MMP* sequences variants are associated with other musculoskeletal soft tissue injuries such as ACL.

The ADAMTS are a family of 19 structurally related zinc-containing metalloproteases that catalyse a range of substrates including proteoglycans [90], several procollagens [90], thrombospondin 1 and 2 (ADAMTS 1) [91] and Von Willebrand factor (ADAMTS 13) [92]. A number of growth factors and hormones are known to modulate *ADAMTS* gene expression [92, 93], and strain-induced tendon abnormalities have been reported to influence the expression of *ADAMTS1* in an ovine model [94].

Studies on the expression of a large number of metalloproteases, including all *ADAMTSs,* in normal and degenerative Achilles tendon have revealed a reduction in *ADAMTS 5* expression in painful Achilles compared with controls [95]. The same study also demonstrated that ADAMTS 7 and ADAMTS 13 were repressed in ruptured Achilles compared with healthy controls [95]. Recently the rs4747096 variant within the *ADAMTS14* gene has been shown to associate with various osteoarthritis phenotypes in Caucasians [96]. Whether this variant predisposes to tendon or ligament injury remains unknown. Although it has previously been suggested that *ADAMTS* gene variants should be considered as candidates for musculoskeletal soft tissue injuries [11], no population-based genetic association studies, to date, have emerged in the literature.

Conclusion

To summarise, genetic association studies have shown that sequence variants within three genes (*TNC, COL5A1* and *MMP3*) co-segregate with chronic Achilles tendinopathy [29, 30, 58, 89]. The variant within the *TNC* gene also appears to co-segregate with Achilles tendon ruptures [30], while sequence variants within the *COL1A1*, *COL5A1* and *COL12A1* genes have been shown to be associated with cruciate ligament ruptures and/or shoulder dislocations [32, 35, 36]. Functional variants within pyrophosphate metabolism genes (*ANKH* and *TNAP*), on the other hand, are associated with rotator cuff tear arthropathy [97]. Whether these variants are directly involved in the development of these musculoskeletal soft tissue abnormalities or in strong linkage disequilibrium with actual disease-causing loci remains to be established. However, all the genes that contain sequence variants shown to be associated with soft tissue injuries to date encode for proteins directly involved in biological processes within tendons and/or ligaments.

In terms of additional molecular mechanisms that may predispose to musculoskeletal soft tissue injury, recent work has demonstrated that certain CpG sites within the promoters of *MMP3, MMP13* and *ADAMTS 4* genes are 'unsilenced' by DNA hypomethylation in osteoarthritic chondrocytes [98]. It has been postulated that these

epigenetic factors may lead to the elevated expression of these proteins in the disease [98]. Accordingly, establishing whether DNA methylation profiles are altered in genes associated with musculoskeletal soft tissue injury would be a future area worthy of research.

In conclusion, using genetic association studies, investigators have started identifying genetic variants which predispose individuals to altered risk of specific acute and overuse musculoskeletal soft tissue injuries. Although it is too early to speculate with respect to specific musculoskeletal soft tissue injuries, the current evidence suggests that different genetic variants may be associated with acute and overuse injuries. Evidence is also starting to emerge that these conditions are polygenic in nature. Research in the area could be accelerated if international consortia, which use high throughput genome-wide association or sequencing technologies on cohorts consisting of larger sample sizes, were established. Prospective follow-up studies are also required to confirm these initial findings, while any proposed molecular mechanism should to be tested using cell culture and other systems. We propose that eventually genetic risk factors will be included in multifactorial models developed to understand the molecular mechanisms that cause musculoskeletal soft tissue injuries. It is conceivable that these models could in the future also be used by clinicians and other professionals to develop personalised training programmes to reduce the risk of injury as well as treatment and rehabilitation regimens for injured athletes.

Acknowledgements

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Innovative Strategies for Treatment of Soft Tissue Injuries in Human and Animal Athletes

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Abstract

Our aim is to review the recent progress in the management of musculoskeletal disorders. We will cover novel therapeutic approaches based on growth factors, gene therapy and cells, including stem cells, which may be combined with each other as appropriate. We focus mainly on the treatment of soft tissue injuries – muscle, cartilage, and tendon/ligament for both human and animal athletes. The need for innovative strategies results from the fact that despite all efforts, the current strategies for cartilage and tendon/ligament still result in the formation of functionally and biomechanically inferior tissues after injury (a phenomenon called 'repair' as opposed to proper 'regeneration'), whereas the outcome for muscle is more favorable. Innovative approaches are urgently needed not only to enhance the outcome of conservative or surgical procedures but also to speed up the healing process from the very long disabling periods, which is of special relevance for athletes.

Injuries of soft tissues of the musculoskeletal system are common and often present as long-time disabling. This situation affects individual persons and even more so professional athletes. Therefore, novel therapeutic strategies need to be developed – in particular based on regenerative medicine and tissue engineering. Regenerative medicine is a multidisciplinary approach to create living, functional substitutes for restoration, maintenance, or improvement of tissue or organ function that has been lost due to injury, disease, age, damage, or congenital defects – either in vitro for subsequent application in vivo, or directly in vivo. This field holds the promise to develop remedies for previously irreparable defects and to solve the problem of organ shortage for transplantation. One important aspect of regenerative medicine is tissue engineering. It includes three main elements: cells, factors or stimuli, and biomaterials. Cells ('grafts') can be autologous or allogeneic and include stem cells where appropriate. Apart from different advantages, choice of donor cells depends on the consideration of disadvantages such as additional trauma during graft harvesting (autograft), infection

or immunorejection (allografts). The factors or stimuli are bio(re)active molecules and may be proteins like growth factors and cytokines, drugs, or others. They can be soluble, insoluble, or immobilized. The biomaterials for tissue engineering, which can be derived from natural (like collagen) or synthetic sources (e.g. ceramics, polymers of lactic and glycolic acid), need to be biodegradable and must fulfill several criteria: biocompatibility, capability of integrating molecules or cells, lack of toxicity and immunogenicity, proper biomechanical characteristics, three-dimensional structure, controlled delivery of factors and many more.

Much work within the musculoskeletal system has been done in bone, which is a highly vascularized tissue. Due to the vascularization and medical progress, healing of bone after fracture often proceeds readily. In contrast, soft tissues like cartilage, tendons, and ligaments are poorly vascularized and heal slowly. And their healing ends with the formation of fibrous, scarry tissue which lacks the original flexibility and biomechanical properties.

In this chapter, we will review the recent progress in the management of musculoskeletal disorders focusing specifically on innovative biological methods ('biologicals') including gene therapy for treatment of injuries of muscle, cartilage, and tendon/ligament – for conventional human patients and also for both human and animal athletes.

Wound Healing and Soft Tissue Repair – Current Status

After injury, all tissue healing results from a sequence of defined and consecutive steps: hematoma formation is followed by acute inflammation, then tissue repair by proliferation of fibroblasts and remodeling take place. Ultimately, in many cases less functional tissue is generated in a process called fibrosis or scar formation as opposed to true regeneration which would restore the original functions on a molecular, cellular, functional and biomechanical level. The different stages of healing are not distinct but overlap and are mechanistically intertwined. Their exact timing depends on the type of injury and the severity of damage.

Lesions of the Muscular System

Skeletal muscle is the largest tissue mass in the body, about 40–45% of total body weight, and allows for voluntary force exertion and locomotion. Lesions can be due to direct trauma (e.g. lacerations, contusions, strains, or surgical resection) or have indirect causes like ischemia or neurological dysfunction. Cancer, infectious disease, heart failure, AIDS can produce a body wasting syndrome called cachexia with severe muscle atrophy. Alternatively, muscular dystrophy denotes mostly heritable neuromuscular disorders that likewise cause muscle wasting.

Small muscle injuries like strains can heal completely, but severe injuries typically result in formation of dense scar tissue that impairs muscle function. Muscle injuries in athletes account for 10–55% of sports-related injuries. This frequency is highly variable and depends mainly on the type of sport. Consequently, repair of injured skeletal muscle is of major concern.

Injuries of Cartilage

Cartilage defects can be divided according to their etiology or morphology. As a result of sports injuries, focal injuries typically occur and therefore predominantly affect younger persons. These defects can be subclassified as chondral (cartilage only affected) or osteochondral (defect penetrates into the underlying bone). In older patients, degenerative chondral changes are prevalent. In contrast to bone, cartilage is a tissue with a poor intrinsic capacity for healing which results from poor vascularization (absence of blood vessels and nerve supply), lack of stem cell availability, and/or low cellular turnover, and therefore the efficiency of tissue regeneration is limited. Whereas some spontaneous but transient repair may occur with osteochondral defects since these allow access of stem cells from the vascularized bone to the lesion, chondral defects do not fully regenerate.

Damage to articular cartilage in the knee is a common problem in sports; meniscal tears due to twisting or compression forces are frequent injuries. The menisci are two semilunar fibrocartilaginous structures between the tibia and femur in the knee joint. They function as shock absorbers, joint stabilizers, and joint lubricators. Only tears in the peripheral third of the menisci can heal since this part is vascularized. A special problem exists in the central avascular portion where most tears occur since this never heals and ultimately develops into premature osteoarthritis due to the poor regenerative capacity of articular cartilage which is a prominent example of hyaline cartilage. Sutures, arrows and staples have been used for surgical procedures, but these are only partially successful in alleviating symptoms and do not prevent arthritis development. Apart from total joint arthroplasty which is associated with surgical risks and a finite life span, other techniques developed for therapy include pharmacological intervention, lavage, shaving, laser abrasion, drilling or microfracture of subchondral bone to promote healing, autologous periosteal and perichondral grafting, autologous or allogeneic osteochondral transplantation, and autologous chondrocyte implantation [1]. Autologous and allogeneic grafts for cartilage now are the most common therapeutic approaches. Intense investigations have studied biomaterials on natural or synthetic basis capable of regenerating cartilage defects but so far no good solution has been identified [2], although some recent studies seem quite promising and will be discussed later in the text [3].

Injuries of Tendons and Ligaments

Tendons which connect muscles to bones and ligaments that connect bones to each other are elastic collagenous tissues. They have a similar composition and hierarchical structure of collagen molecules assembling into ever larger units and their strength is related to the number and size of collagen fibrils. Since tendons and ligaments are similar in many aspects we will, for simplicity, only use the term 'tendon' instead of 'tendons and ligaments' in this chapter.

Tendon and ligament lesions can be caused by injury and trauma but also through overuse and ageing. They account for considerable morbidity both in sport and the workplace, and often prove disabling for several months. Orthopedic surgeons see patients with blown-out knees and aching elbows daily. Successful treatment of mostly tendon disorders is difficult and patients often suffer from the symptoms for quite a long time. Moreover, despite remodeling, the biochemical and mechanical properties of healed tendon tissue never match those of intact tendon, which is a major challenge. Tendon naturally heals but – as described above – this process results in repair only, i.e. in the formation of fibrous, scarry tissue which lacks the original flexibility and biomechanical properties and is characterized by a homogenous distribution of smaller than usual diameter collagen fibers and a higher content of collagen type V and decorin and other proteoglycans. Healed tendon therefore shows reduced performance and exhibits a substantial risk for re-injury. Due to this reason, strategies for a veritable tissue regeneration leading to enhanced elasticity and mechanical performance are highly warranted.

Management of tendon injuries currently follows 2 routes: conservative (rest, rehabilitation and pain relief by drugs) or surgical and the healing outcome of both strategies is similar. Experimental and clinical studies have shown that mobilization of the patient is more beneficial than immobilization [4]. As to surgery, conventional treatment comprises sewing torn tendons in place; those that pull free of the bone can be reattached. However, these surgical repairs do not last for a long time – again, novel therapeutic strategies based on biological approaches are needed. In case of extensive damage, grafts (allo- or autografts) have to be used which either bear the risk of infection and rejection (allograft) or donor site morbidity (autograft).

In addition to the difficulties encountered with tendon regeneration per se, the second major obstacle in surgical repair relates to the enthesis, i.e. the site of connection between tendon and bone which is difficult to reproduce. And, such a restored tendon-bone attachment site is the weak link. The attachment sites of tendons to bone are called osteotendinous junction (OTJ or enthesis) that occur in two different forms, according to their histological appearance. The specialized structure of OTJs prevents collagen fiber bending, fraying, shearing, and failure [5]. A fibrocartilaginous (or direct) enthesis is composed of four zones: a dense fibrous connective tissue tendon or ligament zone, uncalcified fibrocartilage, mineralized fibrocartilage, and bone. The outer border of calcification is indicated by a so-called tidemark with

basophilic nature, similar to the tidemark found in articular cartilage [6]. The fibro-cartilage cells synthesize extracellular matrix that is rich in aggrecan and collagen type II, both of which are typical of articular cartilage. In contrast, a fibrous (or indirect) enthesis lacks the fibrocartilage intermediate zone and is made up of tendon and bone zones only. In any case, full recovery from injury at the junction takes at least 6 months – long for a conventional patient and even longer for a professional sportsman.

Towards Future Innovative Strategies: Animal Models

Despite considerable progress, no optimal solution has been found for the treatment of various sports-related injuries, including muscle injuries, ligament and tendon ruptures, central meniscal tears, cartilage lesions, and delayed bone fracture healing [2]. Therapeutic strategies based on developing novel biological approaches are therefore discussed in the following paragraphs.

Local Delivery of Growth Factors

Growth factors are proteins that stimulate cell division upon binding to their appropriate cell surface receptor(s). One class of growth factors with special relevance to the musculoskeletal system is the transforming growth factor-β family which includes bone morphogenetic proteins (BMPs). BMPs have an important role during bone formation and regeneration and were discovered due to their bone-inducing capacities after implantation at ectopic sites. They are dimeric molecules, most often composed of two identical subunits which transmit their signals predominantly through Smad proteins, a group of related intracellular proteins, from ligand-activated cell surface receptors to the nucleus. Activated R-Smads form heteromeric complexes with the common mediator Smad4 (co-Smad4) that translocate into the nucleus where, in combination with transcription factors, they regulate gene expression. A constitutively active R-Smad – i.e. a Smad protein that is constantly active even in the absence of ligand – can be generated artificially and will be further discussed below.

In general, with growth factor approaches, the major problems are firstly, high doses are needed to be therapeutically efficient which may elicit immune responses, secondly, they easily break down in the body and have very brief half-lives in vivo of only hours whereas a normal healing process takes at least 2 weeks. The use of growth factor proteins to promote healing is severely hindered by the difficulty of ensuring their delivery in minimal yet therapeutically efficient levels to a specific injured site. Various strategies based on scaffolds and biomaterials are therefore being developed (polymers, pumps, heparin, etc.). Alternatively, surfaces of conventional materials may be altered by chemical or physical processes (functionalization by chemical

groups, laser structuring of appropriate materials, etc.). For bone repair, approaches including nonspecific and noncovalent absorption of BMP2 on surfaces are providing even better results. Another approach is to chemically modify titanium prostheses or other materials, if necessary including reactive groups that are able to bind growth factors and to release them slowly. We and others could show that such an approach is able to induce biological activity in model cell lines and in vivo as well, see, e.g. [7, 8]. Despite many successful efforts to solve the problem of long-term delivery by strategies like the one just discussed, many researchers may prefer gene-therapeutic strategies (cf. below) or a combination of both, biomaterials and genetically engineered cells or stem cells.

Gene Therapeutic Strategies

In order to transfer genetic material into the target cell(s), specific gene delivery systems called vectors are necessary. They include appropriate regulatory genetic elements and the gene of interest. Such vectors can be based on viruses or on plasmids. Viruses have elaborate systems for transfer of their genetic material into host cells where they replicate themselves and are finally released to infect new cells. Their properties regarding gene delivery can be separated from the pathological consequences of viral replication by removal of selected viral genes. This not only removes the capacity of unwanted and uncontrolled self-replication but also creates space to insert therapeutic genes. Such replication-deficient viral vectors are produced in special cell lines or by transient transfections that provide the viral genes required for replication and packaging into infectious virus. Since they lack the packaging signals that are only attached to the therapeutic gene, the viral genes cannot be packaged into the ensuing virus. Viral vectors have been generated from a wide variety of viruses and therefore, comprise a range of different properties from which they can be selected as applicable. Several systems allow stable and long-term gene expression. Plasmids (nonviral vectors) or naked DNA, on the contrary, are noninfectious and exhibit minimal toxicity. However, they have several drawbacks since their mode of delivery within cationic liposome complexes, as ballistic particles, by calcium phosphate precipitation, or by electroporation is quite unspecific. Transfer efficiency in general is low and expression of the therapeutic gene takes place for only a limited time due to degradation.

Gene transfer can be performed either in or ex vivo, and both strategies need to be considered depending on the individual application and the most suitable route of administration. In ex vivo gene transfer, cells are genetically modified in culture, selected for successfully modified cells by screening or sorting procedures and then given to the recipient either systemically (mainly the blood) or locally (e.g. intraarticularly). The selection processes make ex vivo strategies highly efficient but autologous cell harvesting is an additional burden for the patient and the subsequent cell

modifications are time-consuming and expensive. A major advantage but simultaneous limitation of this strategy is that in many cases the treatment could or should be patient-specific to avoid long-term immunosuppression. Examples of therapeutic animal studies in the soft tissues reviewed here will be discussed below in combination with cellular approaches.

In vivo gene transfer, in contrast, means that a vector with the gene of interest can be prepared for the treatment of many individuals, which also reduces the cost of application. In addition, such an approach allows the use of nonintegrating vectors that will lead to relatively short-term gene expression in mitotic (dividing) cells but long-term gene expression in postmitotic cells. In the present context, this applies specifically to muscle fibers. As a disadvantage, no selection and screening of successfully modified cells is possible. Such a somatic gene therapy is legal and would be sufficient for athletes' needs. Local injection at the injury site seems to be the method of choice. The major problem is to ensure that the necessary genes are expressed in the right cells.

We will focus on athletes later on in this chapter. Here, the main target of gene therapy (either for enhanced recovery following injury or degeneration or for improved performance during competitions as 'gene doping') is the musculoskeletal system. Systemic delivery in the musculoskeletal system faces special hurdles since cartilage and tendon lack sufficient blood supply. Despite all efforts, gene therapy is almost exclusively experimental medicine, far from routine applications, and much additional preclinical and clinical studies are necessary to prove efficacy and safety [9]. The misuse of this technique for performance enhancement of athletes bears the risk of serious health problems or side effects including death when used in otherwise healthy people without a clinical need.

Cell- and Stem Cell-Derived Therapies Including Genetically Modified Cells

Due to the limitations of pure growth factor or gene therapy, many labs investigate the usefulness of cells. For regeneration, differentiated cells may be helpful as such or by secreting factors ('trophic effects') that stimulate neighboring or recruited cells to induce a healing response, whereas stem cells may be particularly helpful by differentiating themselves into appropriate cell types. Stem cells have elicited broad interest and excitement due to the promise that either embryonic, adult or induced pluripotent stem cells (derived from somatic cells by overexpression of specific factors) could be used for tissue regeneration for injuries where current therapeutic strategies lack efficiency. Adult stem cells open up the possibility to treat diverse diseases by autologous transplantation (i.e. using the patient's own cells after ex vivo expansion), either through local application or through systemic infusion. Alternatively, since human mesenchymal stem cells (MSCs) are thought to be non- or only faintly immunogenic, even the use of allogenic donor cells might become feasible. They have – due to their

possible use in regenerative medicine and tissue engineering – attracted much interest [10]. The transplantation of MSCs into a variety of injured skeletal tissues has been shown to promote healing. In the gene-therapeutic setting, MSCs may be intentionally modified by overexpression of growth or transcription factors to more efficiently conduct their differentiation into appropriate cell types or by secreting factors that stimulate neighboring or recruited cells to induce a healing response. Recently, a subpopulation of human perivascular cells was identified that express both pericyte and MSC markers (one hallmark is expression of the surface antigen CD146, melanoma-associated cell adhesion molecule) in situ, both in vivo and in vitro. The isolated population can be expanded and is clonally multipotent in culture [11]. CD146 positive pericytes can also be serially transplanted to create a hematopoietic environment [12]. These cells seem to be superior to the conventionally isolated MSCs since each single clone is multipotential, and they can be kept in culture for at least 17 weeks or 14 passages [11], whereas conventional MSCs go into senescence already around 6 passages. The use of these stem cells may notably enhance human therapeutic strategies for the musculoskeletal system in the future. Alternatively, the recently described and rapidly expanding field of induced pluripotent stem cells holds great promise of creating patient-specific cells for therapy. Several studies have demonstrated their utility in animal models of human disease like sickle-cell anemia [13] whereas studies in the musculoskeletal system are still missing. However, an inherent tumorigenic potential of these cells has to be closely observed.

As with growth factors, one of the challenges with (stem) cells is to determine the best way to deliver them to their target tissue [14]. To do so, several different biomaterials and scaffolds are in use similar to the ones described for growth factors. One elegant way is to suspend the cells in a viscous fibrin gel which hardens after injection into the body and from which cells can exert their functions. In addition to the mode of delivery, cell transplantation includes more challenges: the way to obtain appropriate cells and to ensure their survival in the recipient.

Exercise and Soft Tissue Regeneration

The musculoskeletal system is particularly subject to mechanical stimuli and loading to properly exert its functions; loss of loading rapidly results in loss of functionality. As a consequence, all components of the musculoskeletal system can be influenced beneficially by exercise and sports and the importance of mechanical stimuli as biological regulators for connective tissue physiology and pathology has been well documented [15–17].

In muscle, exercise may induce satellite cell proliferation and muscle growth [18]. Mechanical stimulation influences metabolic activity, gene expression, and myogenesis as well as fiber alignment [1]. Likewise, many tendon engineering protocols have relied on the application of mechanical load as reviewed, e.g. in reference [19] or [4].

Recent attempts to optimize the functional properties of engineered cartilage have focused on using bioreactors to incorporate mechanical loading environments in vitro to mimic in vivo conditions [1]. In this context, dynamic deformation, hydrostatic pressure, fluid flow, and shear stress were tested, and beneficial effects of mechanical loading on cartilage constructs have been widely reported.

Examples of Novel Treatment Strategies in Animal Models

Muscle

Muscle regeneration occurs early in the healing process. It usually begins 3–5 days after injury, peaks around the 2nd week, and declines thereafter [18]. Various growth factors like insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), or nerve growth factor (NGF) can improve muscle regeneration during these early stages [18]. Local injection of IGF-1, bFGF and – although to a lesser extent – NGF after injury increases the number and size of regenerating myofibers in different mouse injury models. Amongst these, IGF-1 has the greatest beneficial effects since it can increase the efficiency of muscle regeneration and muscle strength in vivo [20]. The so-called Schwarzenegger mouse which is transgenic for IGF-1 presents with enormous muscle generation, i.e. 20–30% greater than in normal mice. In addition, these mice live longer and recover more quickly from injuries [21].

One potential advantage associated with human recombinant growth factors in the treatment of muscle injuries is the ease and safety of the injection procedure that, however, requires a high amount of factors necessary due to rapid clearance by the bloodstream.

The intramuscular implantation of myogenic cells is an approach to develop a therapeutic tool for myopathies, i.e. for diseases with a genetic background [22]. Most studies have been performed on animal models for and patients with Duchenne's muscular dystrophy. For treatment of skeletal muscle, differentiated muscle cells cannot be used because they can neither be properly manipulated nor implanted. Instead, cellular therapy of muscle relies on satellite cells which are the muscle stem cells located on the outer side of muscle fibers, between the basal lamina and plasma membrane. After injury, these cells are triggered to divide and to recapitulate the myogenic events: formation of myoblasts, myocytes, and finally of multinucleated myotubes and muscle fibers. Satellite cells are easily amenable to therapeutic manipulations since they can readily be isolated from muscle biopsies and maintained in culture.

The main factor during intramuscular cell injections to consider is the fact that injected cells fuse mainly with the myofibers damaged by the injection trajectories. As a consequence, cell implantation protocols should include 'high density' injections: 100 cell injections/cm^2 may serve as a guideline [22] but may entail different risks like developing a compartment syndrome in muscles that are enclosed in a rigid osteofacial space. To ensure the long-term survival of myoblast allografts [22], an

appropriate immunosuppression would be required. As a consequence, like in the case of gene therapy, such strategies should currently be limited to patients suffering from severe muscular diseases but should not be applied to athletes to speed up a healing process.

The potential therapeutic value of MSCs for muscle repair has been a controversial issue. However, recent studies show that GFP-labeled human MSCs were able to undergo myogenesis upon transplantation. Histology revealed mature muscle characteristics in most GFP-positive myofibers. In addition, some of the cells even seemed to become Pax7-expressing satellite cells [23]. Most recently, the human perivascular cells expressing both pericyte and MSC markers including CD146 have convincingly demonstrated a high potential for muscle fiber generation. This was true not only for cells isolated from skeletal muscle but also for perivascular cells obtained from other donor sites [11].

Readers interested in learning more about therapeutic strategies for muscle injuries might want to see the excellent review by Huard et al. [2].

Cartilage

In contrast to other tissues of the musculoskeletal system, therapeutic approaches using growth factors alone (i.e. in the absence of cells) are not often investigated since they have been even less promising than strategies involving cells. In principle, fully differentiated chondrocytes would be the ideal cell candidate for cartilage repair. Autologous chondrocytes derived from a minor load-bearing area and injected under a periosteal flap can repair full-thickness chondral defects. This procedure (trade name: Carticel™, Genzyme Biosurgery) is the only FDA-approved cell-based therapy for cartilage repair [1]. Apart from being costly and nonconclusively superior to other methods, this autologous technique suffers from potential donor side morbidity, limited availability of chondrocytes, and dedifferentiation of the isolated chondrocytes in monolayer culture. MSCs as a potential alternative to chondrocytes have not proven to be very successful yet. Specifically, their potential to differentiate into chondrocytes in response to transforming growth factor-β is quite limited as a panel of studies have shown.

In the menisci, there are special cells with fibroblast- and chondrocyte-like properties called fibrochondrocytes. Recent evidence has pointed to the potential use of MSCs for meniscal regeneration: GFP-labeled rat MSCs were seeded in fibrin glue and used to treat meniscal defects. These cells were detectable until 8 weeks after transplantation and promoted repair [24]. However, for a successful meniscal therapy, further optimization of regenerative conditions and factors is necessary. Yet another challenge resides in the fact that a functional meniscal tissue must exhibit notable anisotropic mechanical properties resulting from a highly oriented underlying extracellular matrix [1]. In order to generate a scaffold which has controllable, anisotropic properties and mimicks meniscal extracellular matrix fiber alignment to direct fibrochondrocyte orientation, electrospinning technology was used [25]. This technique

is based on a biodegradable polymer and results in cartilage with about 20% of the strength of native cartilage but with quite small pores that are difficult for chondrocytes to penetrate [3].

A variety of other natural and synthetic biomaterials have also been investigated. Moutos et al. [26] have created a scaffold which leaves more space for the cells than the electrospinning material. In particular, a different biodegradable material was used in a novel three-dimensional weaving technique which gives compressive, tensile, and sheer strength highly similar to native cartilage, but it degraded too rapidly in vivo. Using another polymer as the basis, the group hopes to go into clinical studies in 2010 [3]. Chiang et al. [27] seeded chondrocytes into gelation microbeads mixed with a collagen type I gel. This enhanced repair in a porcine cartilage defect model, and the chondrocyte phenotype was maintained better than without the carrier. Many more scaffolds and matrices have been under examination [reviewed, e.g., in 1, 28] but so far they have shown variable effects on cell behavior and function.

Viral vectors have been used in several studies to deliver therapeutic genes to chondrocytes in vitro [2]. In addition, these modified cells were applied in collagen gel or organ tissue explants. In vitro delivery of genes for BMP2, IGF-1, and TGF-β to articular chondrocytes has been shown to increase proteoglycan synthesis by the cultured cells [see, e.g., 29, 30]. After retroviral overexpression of BMP-7 in MSCs and cell delivery on a polyglycolic acid scaffold, a rabbit osteochondral articular cartilage defect showed improved healing [31].

In conclusion, there is still a long way to go and develop the optimal strategy and cell source for cartilage repair especially with respect to functional and biomechanical behavior.

Tendon

Within the past years, the American Food and Drug Administration has approved BMP2 and BMP7 for human application, mainly for bone repair. However, BMPs may also prove efficient for cartilage and tendons. BMP12, e.g., was tested for healing of rotator cuff injuries in sheep [32] using two modes of long-term delivery: a paste and a biodegradable collagen sponge to release BMP12. In treated sheep, shoulders were three times stronger (1.8 kN as compared to 0.6 kN) than in sham-operated animals. However, this strength was much less than for a normal, nonruptured shoulder (3.7 kN).

As mentioned in the introduction, one major obstacle in tendon ruptures is the fact that tendon-bone junctions cannot easily be reconstructed to provide secure healing: Especially fibrocartilaginous osteotendinous junctions do not regenerate properly and all attempts for generation of such an enthesis with four zones have failed so far and resulted in fibrous enthesis only [33]. And, an enthesis in the wrong anatomic location entails limited mechanical strength. Therefore, one recent study investigated the effect of rhBMP-2 as compared to noggin, a potent BMP inhibitor. Indeed, BMP2 demonstrated a strong, positive dose-dependent effect on osteointegration at the

tendon-bone junction. In contrast, noggin decreased osteointegration. Further studies are necessary to investigate the potential clinical application of enhancing healing and decreasing recovery time with BMPs [34]. A recent study by Hashimoto et al. [35] used application of BMP2 on native rabbit tendon which resulted in ossicle formation including a cartilage region but such an approach requires two surgeries, one to ossify a graft tendon, and a second one to reconstruct the injured site.

Delivery of therapeutic genes has also been investigated for a beneficial use in de novo tendon formation and tendon repair. Several studies were conducted with reporter genes based mainly on adenoviruses in order to assess the overall feasibility of gene transfer into tendon tissues. These studies reveal that direct administration of adenoviruses is possible but may not be the optimal choice for tendon repair since their dense extracellular matrix apparently interferes with efficient infection [10].

In principle, the primary sources of cells for tendon regeneration are currently expected to be tenocytes, MSCs, or tendon stem cells. Tenocytes, derived from tendon progenitor cells, secrete large amounts of collagens which aggregate into basic tendon fibrils. Two major problems with the tenocyte as a therapeutic vehicle, however, arise: Firstly, tendons contain relatively low amounts of cells resulting in limited availability; secondly, they do not proliferate well in culture, dedifferentiate, and lose the characteristic tenocyte morphology [36].

Several studies using MSCs have been conducted and strategies for a successful MSC-dependent repair of tendon tissue have been described. Repair of the Achilles, rotator cuff and patellar tendons has been reported. De novo tendon-like tissue formation was observed after 3 months [37]. In another study, MSC-collagen composites were used for tendon repair of the patellar tendon. In this study, a higher capacity to resist maximum stress than natural repair was reported [38].

Our group could show that a murine MSC-like cell line genetically engineered to express BMP2 and a biologically active Smad8 variant differentiates into tendon-like cells. The engineered cells demonstrated the morphological characteristics and gene expression profile of tendon cells both in vitro and in vivo. In addition, following implantation into an Achilles tendon partial defect, the engineered cells were capable of inducing tendon regeneration [39]. More recently, we could show that by appropriate manipulation of BMP2 and constitutively active Smad8 expression levels the formation of either pure tendon-like tissue or, alternatively, tendon attached to osteotendinous junctions, is possible. Interestingly, C3H10T½ cells generate fibrocartilaginous entheses, whereas human MSCs give fibrous entheses [Shahab-Osterloh et al., unpubl.]. Especially the creation of fibrocartilaginous entheses in vitro would be therapeutically highly desirable.

In 2007, researchers reported that they successfully isolated small numbers of cells with stem cell characteristics from the extracellular matrix of both mouse and human tendons – so called tendon stem cells. Their study showed that adult tendons contain a rare subset of stem cells that can be isolated, grown in the lab, and then used to generate tendon-like tissues at least in mice [40]. Future studies will have to

show whether tendon stem cells may be useful in human therapies – one question to answer will be whether these cells can be made available in high enough numbers in a reasonable period of time.

Examples of Therapeutic Strategies in Athletes

Sports injuries usually involve tissues that display a limited capacity for healing. Experience is still limited in human athletes due to concerns about doping [this is discussed by Wells, this vol., pp. 166–175]. However, in the treatment of athletic injuries, growth factor, gene and or cell therapies may induce more rapid healing or result in less side effects from the repair process, such as adhesions and fibrosis. Studies in this field are limited and there is no clear evidence supporting long-term enhanced athletic performance arising from such therapies in animal studies [41]. For instance, expression of growth and differentiation factor-5 in the region of a joint-associated tendon repair improved the range of movement but did not change the (limited) biomechanical properties in a mouse model [42].

Tendon

The anterior cruciate ligament (ACL) consists of a tough band of tissue that connects the thighbone to the shinbone. It stabilizes the knee and enables its motions, including not only stretching but also turning. A torn ACL does not heal well even if surgeons manage to sew the ends back together, which means that athletes cannot compete for several months – and especially for a professional athlete, this is a highly unpleasant situation. Conventional therapy means that often the patellar or hamstring (i.e. semitendinosous plus gracilis) tendon of the patient is used as an autograft to replace the torn ACL. This, however, entails significant donor site morbidity and often proves to be even more disabling than the original defect. Grafting of the central third of the patellar tendon-bone may also lead to patella baja, arthrofibrosis, adhesion to the fat pad, and patellofemoral pain which is hardly helpful for normal patients and even less for sportsmen. Allografts have seen limited use except in revision surgery or for multiple ligamentous injuries [4].

Many studies have been conducted in animals. In particular, there are many similarities between the weight-bearing tendons of the horse and those of the human athlete (e.g. Achilles tendon) in their hierarchical structure and matrix organization, their function and the nature of the injuries sustained. The horse is to be considered as a very special and precious animal athlete suffering often from strain-induced tendon or ligament injuries, resembling consequences of athletic endeavor in humans. Both in horses and in humans, this results in high morbidity and often prevents return to the original performance. Many veterinarians, therefore, are considering

therapeutic strategies based on MSCs which, similar to humans, can be preferentially harvested either from equine bone marrow or adipose tissue for treatment of tendon ailments. For horses, MSCs have been applied for the surgical treatment of subchondral bone cysts, bone fracture repair, and cartilage repair [43], and prominently, for tendon injuries. These studies at the same time not only help to cure the horses but also advance our understanding of orthopedic treatment options and enhance translational research into the clinics.

Muscle

Depending on the type of sport, muscle injuries constitute between 10 and 55% of all injuries sustained by athletes [2]. Of relevance for muscle-targeted therapies and gene doping, promoters derived from muscle creatinine kinase, smooth muscle cell α-actin and myosin heavy and light chain have been used in cell culture and animal model studies to achieve muscle-specific expression of recombinant proteins. At present, such strategies should be applied to patients suffering from severe muscular diseases but not to athletes to speed up a healing process because there are many more risks than potential benefits.

Cartilage

As alluded to in the preceding sections of this chapter, the current state of research in cartilage engineering of human patients is not yet entirely promising. However, improvements in culture conditions and scaffold materials, incorporation of bioactive factors, recognition of the role of mechanical stimulation in cartilage formation and novel techniques increasingly contribute to improvement of not only the biological behavior but also the mechanical properties [28]. The 20% of the strength of the native cartilage that has been obtained by several groups is still far from the clinical needs of patients. Such innovative strategies will certainly be of benefit for athletes under the above-mentioned precaution that they will not be abused for doping and give them an illegal advantage over their competitors.

Acknowledgements

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Gene Doping: Possibilities and Practicalities

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Abstract

Background: Our ever-increasing understanding of the genetic control of cardiovascular and musculoskeletal function together with recent technical improvements in genetic manipulation generates mounting concern over the possibility of such technology being abused by athletes in their quest for improved performance. Genetic manipulation in the context of athletic performance is commonly referred to as gene doping. **Methods:** A review of the literature was performed to identify the genes and methodologies most likely to be used for gene doping and the technologies that might be used to identify such doping. **Results:** A large number of candidate performance-enhancing genes have been identified from animal studies, many of them using transgenic mice. Only a limited number have been shown to be effective following gene transfer into adults. Those that seem most likely to be abused are genes that exert their effects locally and leave little, if any, trace in blood or urine. **Conclusion:** There is currently no evidence that gene doping has yet been undertaken in competitive athletes but the anti-doping authorities will need to remain vigilant in reviewing this rapidly emerging technology. The detection of gene doping involves some different challenges from other agents and a number of promising approaches are currently being explored.

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The Concept of Gene Doping

Gene doping is the term applied to the use of genetic manipulation to improve athletic performance. The World Anti-Doping Agency (WADA) prohibits this practice as specifically 'the non-therapeutic use of cells, genes, genetic elements, or the modulation of gene expression, having the capacity to enhance athletic performance'.

Concerns over the possibility of gene doping have been raised following a number of demonstrations of increased running endurance and muscle force after genetic manipulation in rodents. Gene doping offers a number of potential advantages over conventional doping. Firstly, as the protein is made within the body of the treated individual, it will in many cases be indistinguishable from the normal endogenous protein. Secondly, as production of the protein will be steady state, a lower level of

continuous expression will likely achieve the same effect as the intermittent high doses given exogenously. Thirdly, in many cases of potential gene doping, the vector and gene product will remain largely in the treated tissue such as the muscle and so will only appear at very trace amounts, if at all, in the routine test samples of blood or urine. All of these properties will make it harder to detect gene doping compared with conventional doping and so increase the allure for athletes who wish to gain an unfair competitive advantage.

Candidate Genes for Improving Athletic Performance

A number of genes have been associated with improved athletic performance, either as the results of identifying individuals with genetic changes associated with enhanced strength or endurance, or through the demonstration of improved performance in genetically modified (GM) animals. Examples of the former include the marked muscle hypertrophy reported in a myostatin null infant [1] and the Finnish cross-country skier Eero Mantyranta who had a natural mutation in his erythropoietin (Epo) receptor gene that increased his haemoglobin levels and so improved endurance [2]. There are many more examples of genetically manipulated animals and some of these are listed in table 1. Of particular interest are those genes that are expressed in the target tissue and will therefore not be present in the circulation. These include transcription factors that regulate the fibre type pattern and oxidative capacity of muscle (e.g. peroxisome proliferator-activated receptor-γ – PPARγ and the coactivators PGC1α and PGC1β).

The reader should be aware that many of the genes identified as offering potential athletic advantage have to date only been reported from studies with transgenic animals. In such transgenic studies, the genetic manipulation occurs in the early embryo such that the modification is carried in every cell of the animal, although depending on the elements controlling expression a gene may only be active in specific tissues or at specific times. In most cases, the genetic modification is expressed during development and the organism may adjust patterns of expression of other genes to accommodate this genetic difference. In contrast, genetic modification in the adult, as might be practiced with gene doping, has not been performed for many candidate genes and so the potential benefits are unclear. For example, modification of muscle phenotype by a transgene may influence motor neuron development to ensure a match but will the same happen following gene transfer into adult muscle? There is one study that has partly addressed this question. Lunde et al. [3] introduced a constitutively active form of a transcription factor, PPAR-δ, which drives oxidative fibre expression, and showed that somatic gene transfer induced similar shifts in fibre-type and oxidative capacity as the transgenic experiment performed by Wang et al. [4] when the lower efficiency gene transfer in the adult was taken into account. It remains to be shown if the same is true for other activators of the same pathway and if such gene transfer changes muscle physiology.

Table 1. A list of examples of genes shown to improve athletic performance in GM animals

Gene	Experimental system	Outcome	Reference
IGF-1	Transgenic mice	Muscle hypertrophy	[32]
IGF-1	AAV-mediated gene transfer in mice	Prevented age-related loss of muscle mass	[33]
IGF-1	AAV-mediated gene transfer in rats	IGF-1 acted synergistically with resistance training to increase muscle power output	[34]
Growth hormone releasing hormone	Plasmid electroporation of muscle in mice, dogs and pigs	Increased muscle mass and other anabolic responses	Reviewed in [35]
PPAR-δ	Transgenic mice	Doubled running endurance compared with wild-type mice	[4]
PPAR-δ	Gene transfer into rat muscle (plasmid electroporation)	Increased IIa and decreased IIb fibres following acute gene transfer	[3]
PGC-1α	Transgenic mice	All muscle type 1 with high fatigue resistance	[36]
PGC-1β	Transgenic mice	High proportion type IIX increased endurance at high work loads	[37]
Phosphoenolpyruvate carboxykinase	Transgenic mice	Substantially enhanced aerobic exercise capacity	[38]
Myostatin deletion	Knockout mice	Muscle hypertrophy	[39]
Myostatin blockade	Hydrodynamic limb vein injection of AAV8 myostatin propeptide (blocks myostatin) in the dog	Muscle hypertrophy in the treated limb	[13]
Epo	Electroporation of plasmid into mouse and non-human primate muscle	Increased hematocrit	[40]
Epo	Intramuscular AAV into non-human primate	Rapamycin-regulated expression gave control of hematocrit	[16]

For further details on these and other genes, please see the excellent review by Baoutina et al. [31].

Improving Recovery from Injury

Apart from improving athletic performance, gene doping can potentially be used to accelerate recovery from injury. The genetic potential has again been demonstrated in GM animals either by transgenesis or following somatic gene transfer. While it would

be wrong to deny athletes approved genetic therapies when they become available, there is the potential for athletes to gain a competitive advantage long after the injury has been resolved.

A key feature required for rapid and efficient repair of a bone, joint or tendon defect is an effective blood supply [5]. Neo-vascularisation can be promoted by a number of factors such as vascular endothelial growth factor (VEGF). In addition, other growth factors, such as bone morphogenic protein, activate osteoblasts improving the rate of bone repair. Thus delivery of the DNA encoding such growth factors will enhance repair and so speed recovery from injury. While this would be permitted on therapeutic grounds, it is possible that expression of some of these factors could enhance the strength of bones and tendons, thus giving an advantage to competitors using these techniques, particularly in the strength disciplines like field athletics and weightlifting. Consequently, there is a possibility that such therapies would be used to boost performance in the absence of injury. The current state of the art in orthopaedic gene therapy has recently been reviewed by Evans et al. [6]. They conclude that although the technology is very promising, clinical trials are constrained by safety fears as most orthopaedic conditions are not life threatening unlike many of the other targets for gene therapy. Hence the clinical potential of such methods is unproven.

Muscle repair after injury might also benefit from improving the blood supply with molecules such as VEGF, although a recent review by Yla-Herttuala et al. [7] reveals relatively disappointing results from human clinical trials in cardiovascular disorders to date. IGF-1 overexpression enhances muscle repair in a rodent model of muscle injury [8]. Again both of these treatments for muscle damage also have the potential to increase athletic performance.

Current Practicalities in Gene Delivery

The key three barriers to the successful clinical application of gene therapy are delivery, delivery and delivery. The same problem applies to gene doping. In order to treat conditions such as Duchenne muscular dystrophy (DMD), a lethal muscle wasting disease seen mostly in boys that affects all muscles, a regional or systemic delivery method is necessary for clinical benefit.

Gene transfer can occur either ex vivo or in vivo. In the former case, cells of the individual are grown in culture, genetically modified, selected and then transplanted back into the donor. In the latter, the gene transfer is applied directly to the individual as a local, regional or systemic application. The ex vivo approach requires specialised facilities for cell culture and is labour intensive, but has the advantage that the level of expression of the gene in the GM cells can be assessed. In contrast, the in vivo approach, while much simpler, offers less control over the level of gene transfer and subsequent expression. In both cases, it is necessary to use a system for gene delivery, commonly referred to as a vector.

Heavily modified viruses are used as gene vectors in order to benefit from the natural mechanisms for the delivery of genetic material into cells, an essential part of the viral life cycle. By deleting specific viral genes, replication and consequent cell damage or death is prevented. This also provides space to accommodate the gene(s) of interest. In pre-clinical studies, the most commonly used vectors have been based on retroviruses (including lentiviruses), adenoviruses and adeno-associated viruses (AAVs). Retroviral vectors integrate into the host genome, thus providing a permanent genetic change whereas adenoviral and AAV-based vectors exist as an episome in the nucleus and are lost following cell division. However, for post-mitotic tissues such as muscle the effect of gene transfer is semi-permanent unless the tissue is damaged. Pre-clinical studies in experimental animals ranging from mice to non-human primates have demonstrated that AAV-based vectors can be particularly efficient at gene delivery to muscle and, at least in the mouse, systemic delivery to all muscles is possible [9]. A major current drawback to such vectors is that immune responses to the viral capsid proteins prevent administration of a second dose, although the use of other non-cross-reacting serotypes can circumvent this problem. Alternatively, a regimen of short-term (approximately 6 weeks) immunosuppression at the time of administration may allow repeated treatments. These regimes are currently under intensive investigation to enable the use of such vectors in the clinical treatment of conditions such as DMD.

The alternative to the use of viral vectors is the non-viral system based on purified circular DNA plasmids grown in bacteria. These are very easy to produce and are the basic tool of any molecular biology research project. The delivery of such plasmids to cells generally requires the use of transfection reagents that condense the DNA and encourage uptake by endocytosis into the cells. It is possible to avoid such agents by using physical methods to create transient pores in the cell membrane and so allow entry of the naked plasmid DNA into the cell. These physical methods can be particularly effective in vivo, and techniques such as electroporation [reviewed in 10] and hydrodynamic delivery (reviewed in [11]) can rival the efficiency of viral vectors.

Regardless of the vector system, there are some generic challenges for gene delivery. Local intramuscular injection leads to very limited spread of the vector and so is only suitable where the product will be secreted by the muscle and enter the general circulation (for example Epo or growth hormone). Gene delivery to the whole body does not currently appear to be a realistic option but gene delivery to muscle using perfusion of temporarily isolated limbs (using a tourniquet) has been successful in several animal models using both viral and non-viral vectors [e.g. 12–13]. Expression of the gene can be restricted to muscle by use of a muscle-specific promoter.

A major consideration for the potential gene doper is the ability to control the level of expression of the transferred gene. This will be important as uncontrolled expression may lead to decreased rather than increased performance (see below). As noted above, ex vivo gene transfer offers assessment of the level of gene expression

and so one option would be to transfer a given amount of GM cells as a free-standing implant that could if necessary be removed at a later date. Indeed, such an implant can be protected from the action of the immune system and so would not need to utilise cells from the body of the recipient [14].

In the case of in vivo gene transfer, there are drug-inducible systems that would allow athletes to regulate the amount of gene expression. These include the tetracycline, rapamycin and oestrogen regulated systems [e.g. 15–17]. These not only allow control over any harm associated with excessive levels of expression but also provide a method for avoiding doping detection in the case of systemically secreted proteins.

Potential for Harm

Gene doping carries risks associated with the gene delivery and the consequences of gene expression. Large doses of viral vectors risk triggering a massive release of cytokines by the innate immune system and this has proved fatal for one patient in a gene therapy trial [18]. Plasmid DNA can also activate the innate immune system via specific sequences that are recognised as bacterial, and this leads to inflammation and fever. Recent developments in non-viral vector design have largely removed these sequences, which has lead to significantly less interaction with the innate immune system and increased longevity of expression.

Growth hormone and insulin-like growth factor 1 (IGF-1) are both potent mitogens and anti-apoptotic agents and so overexpression following gene transfer carries an increased risk of oncogenesis [reviewed in 19]. Similarly, overexpression of VEGF and other angiogenic factors might lead to increased vascularisation of developing solid cancers and thus could promote tumour growth.

Increasing the number of red blood cells by overexpression of Epo also carries safety risks. An increased haematocrit makes the blood more viscous and increases the load on the heart. This in turn can lead to blockage of the microcirculation including stroke and heart failure. In addition, the production of Epo following gene transfer has been reported as causing autoimmune anaemia in macaques [20, 21].

Detection of Gene Doping

The conventional approach to the detection of doping is to identify a specific banned substance or one of its metabolites using highly sensitive systems such as gas chromatography and high-resolution mass spectrometry. This approach is however limited in that chemical modifications can evade the test, as was seen with the designer anabolic steroid, tetrahydrogestrinone. Consequently, there is increasing interest in

methods that can detect the action of a range of doping agents before trying to focus on detecting the specific substance.

One of the challenges facing detection of gene doping is that only body fluid samples are likely to be routinely available. Thus, it will not be possible to take solid tissue samples to look for evidence of genetic modification. Direct detection in blood may be possible in some cases as circulating proteins expressed following gene transfer into ectopic sites show differences in post-translational modification. This was demonstrated for Epo [22] as well as a recombinant form of factor IX [23] following gene transfer into skeletal muscle. It may also be possible to detect minute traces of gene transfer vectors using highly sensitive polymerase chain reaction-based techniques such as the recently described single-copy primer-internal intron-spanning PCR procedure described by Beiter et al. [24]. The evidence to date [reviewed in 25] shows that the window for detection of vector after administration is relatively small, particularly for non-viral vectors. Intramuscular injections leave relatively little trace of vector in the blood and one solution would be to take fine needle aspirates of muscle. With real-time PCR this seems sufficiently sensitive to pick up evidence of genetic manipulation [26]. However, even this is unlikely to be reliable in the case of local intramuscular administration and such an invasive technique seems unlikely to be widely accepted.

Where regulated promoters are used, it may be possible to detect the activating molecule such as tetracycline, rapamycin or tamoxifen. Alternatively, it may be possible to detect evidence of prior administration of viral vectors by looking for evidence of an antibody response to the virus. However, both of these approaches would be subject to legal challenge either under approved medical use or inadvertent exposure to infectious agents.

Another approach is to use indirect techniques to demonstrate potential gene doping by looking for the consequence of the genetic manipulation such as changes in patterns of target gene expression (transcriptomics), proteins (proteomics) or their metabolites (metabalomics). However, these methods would require the establishment of normal standards and some individuals are likely to fall outside of a normal range as a consequence of natural genetic variation. Indeed, the problem of ethnic and individual variation has been highlighted by assessing the metabolism of testosterone [27]. A solution to this problem would be the use of an athlete's endocrinological passport based on the results of repeated tests over time [28] as has been done for "blood passports" for the detection of blood doping [29]. This would provide testers with a lifelong 'biological fingerprint' of competitors to compare drug-test samples against. This passport concept has been in development with WADA since 2006, but it is far from clear if such a system could be widely implemented.

There are several excellent in-depth reviews of specific aspects of the detection of gene doping that should be consulted for further details [e.g. 25, 30].

Current and Future Perspectives

There is no evidence currently that gene doping is being practiced by athletes, but one of the major lessons of the tetrahydrogestrinone scandal and subsequent revelations is how easy it is for athletes to escape detection unless the testing laboratories know what to look for. Thus, quite reasonably there is a concern that gene doping will be used in the near future. The review of current technology presented here suggests that effective gene doping is currently unlikely, but we live in a rapidly changing period of biotechnology and new developments can occur quite suddenly. It is therefore quite right and proper that the authorities such as WADA take this subject seriously and invest in developing potential screening technology, as much to deter athletes from experimenting as to catch those abusing the system.

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Genetic Testing of Athletes

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Abstract

This chapter addresses the potential use of genetic tests to predict performance and/or risk of exercise-related injury or illness. Various people may wish to conduct a sport-related genetic test on themselves, or on another person, for a variety of reasons. For example, an individual may seek personal genetic information to assist with choosing their own sporting participation or career. A sports coach may wish to test young team members to assist in selection for a professional career or to individualise training. A physician may want to predict risk of injury or illness in an athlete and advise regarding selection or preventative measures. An insurance company may seek to estimate risk of career-threatening injury or illness to an athlete based partly on genetic information. Despite the commercial availability of some genetic tests today, the evidence currently available suggests that few of these or similar scenarios are scientifically justified – the genetic tests available at the moment are simply not powerful enough to inform important decisions in sport. Furthermore, there are many challenging ethical issues to be addressed regarding genetic testing of athletes. The imposition of genetic tests on individuals by third parties, and particularly the imposition of genetic tests on young people, is potentially susceptible to abuse. There should be considerable further debate regarding these issues so that the tests already available, and the more powerful ones that are likely to emerge as knowledge and genetic technology improve, are only used in acceptable ways.

The focus of this chapter is on the potential use of genetic tests to predict performance and/or risk of exercise-related injury or illness. Some earlier chapters of this book, including chapters by Yang et al. [pp. 88–101] (regarding the ACTN3 R577X polymorphism), Pomeroy et al. [pp. 110–135] (exercise, health and genes) and Collins and Raleigh [pp. 136–149] (regarding soft tissue injuries), have already alluded to the notion of testing athletes or other individuals for sport- and exercise-related traits. However, this chapter will deliberately focus on both the scientific and ethical issues regarding the validity, utility, practicality, and ethics of genetic testing of athletes at the current time.

There are already several commercial operations that offer 'mail order' or 'direct-to-consumer' genetic testing of polymorphisms apparently associated with

physical performance capacity or exercise-related health. We believe the first commercial operation began in Australia in 2004 and is marketed as the 'ACTN3 Sports Performance Test^{TM}' [1]. Other commercial operations we are aware of are currently (January 2009) available at 'atlasgene.com', 'mycellfdnafitness.com' and 'cygenelabs.com', some of which claim to test multiple genotypes. The availability of such tests for virtually anyone to access is certainly an interesting development and probably heralds a new era of genetic testing for all manner of individual characteristics. Such tests may become more widely requested because recent legislation in the US ensures that consumers do not have to fear insurance or employment discrimination on the basis of genetic test results [2]. However, significant debate is ongoing regarding the science that is purported to underpin such tests. In addition, there are wider ethical concerns regarding issues such as the confidentiality of data, the need for counselling when interpreting personal genetic data, the use of genetic data in assessing insurance risk, etc. We will address such issues in this chapter.

Is There a Fundamental Difference between Genetic Tests and Non-Genetic Tests?

The film 'GATTACA' (a science fiction film directed by Andrew Niccol about a society where genetic testing is used to determine social class, careers and partners of its citizens) paints a worst-case scenario about discrimination based on the results of genetic testing. 'GATTACA' highlights the concerns that some people have about the potential misuse of genetic testing at a future time when meaningful genetic tests for intellectual or physical ability are available.

Discrimination on the basis of partially inherited factors is already common in sport today. For example, when strength is measured to identify children that have a talent for weightlifting or to select the national weightlifting team. So does it matter whether talent is identified by genetic or by non-genetic tests? Is there a fundamental difference?

To give an example, one could rightly ask 'what is the difference between a performance test that measures a variable that predicts ~2% of an individual's muscle power as opposed to the commercially available ACTN3 R577X genetic test which measures something very similar?' The term 'genetic exceptionalism' is sometimes used in this debate meaning that genetic tests are special and thus require specific legislation that is different than that for other biological tests [3]. In their analysis, Green and Botkin [3] focus on medical tests and conclude that 'no clear, significant distinctions between genetic and non-genetic tests justify a different approach […]' although they state that genetic tests may lead to stigmatisation, family discord and psychological stress, but much of this also applies to some non-genetic tests such as HIV tests.

The working party that developed the BASES position stand on 'Genetic Research and Testing in Sport and Exercise Science' [4] identified two fundamental differences between genetic and non-genetic (performance) tests, and we add a third in this chapter:

1   The information gained from a genetic test does not change with age, whereas the information derived from a traditional performance test, and consequently the predictive quality of that test, does change with age.

2   Not all the predictions that can be made with a genetic test may be known at the time when the genetic test is conducted.

3   Genetic tests have more implications for relatives and partners than other tests. These three points are now discussed in more detail:

1   The genome of an individual and the variations therein are constant throughout life (there are exceptions not relevant for this argument). Consequently, genetic tests can be performed as soon as the DNA of an individual can be obtained – even before birth. This is fundamentally different from other tests such as a lactate test where the information derived from the test, and consequently its predictive quality (for example, the ability to predict Marathon running ability), depend very much on the age when this test is performed. This difference is important because it opens up possibilities for the misuse of genetic tests, especially when used before birth or in minors. One worst-case scenario could be that these tests are used to select embryos on the basis of sporting, intellectual or other abilities (a new eugenics). Also, with young children such tests could be used to make decisions for or against a sporting career.

2   Both genetic and non-genetic performance tests may later be linked to other variables such as disease. We think that this risk is higher for genetic tests than for other performance tests. However, common performance-related variables have also been linked to disease: For example, muscle strength has recently been inversely associated with mortality [5], which means a hitherto unanticipated higher risk of death for individuals who had previously performed poorly in a strength test. However, our opinion is that the risk of an unanticipated disease link is probably higher for genetic tests than for other biomedical tests. One past example is a polymorphism for the apolipoprotein E gene (which encodes a protein important for lipid transport). This gene occurs in three polymorphic forms: APO E2, E3 and E4. The APO E4 variant was initially shown to be associated with limited differences in lipid profile but only later with late-onset familial Alzheimer's disease [6]. Thus, individuals who had previously been tested for a genetic variant that was associated with their lipid profile now retrospectively had information about their likelihood of suffering from the more severe Alzheimer's. How can we deal with the risk of unanticipated future links of genetic test results to severe disease? A pragmatic solution would be to make mandatory genetic counselling necessary so that individuals are aware of this risk before deciding whether to proceed or not with a genetic test.

3   A third difference is that genetic tests have more direct implications for relatives than other tests. As Green and Botkin [3] point out, this is also the case for some non-genetic tests. For example, a positive HIV test result may not only be devastating for the individual but has also far-reaching implications for relatives, current

and previous partners. However, genetic tests are always also predictive for close relatives and this should be taken into account when performing genetic tests. Again, mandatory genetic counselling could be useful to at least ensure that individuals understand the implications of genetic test results for their relatives.

The first difference we have highlighted in particular, and its resultant consequences such as the potential selection of embryos, warrant the use of the term 'genetic exceptionalism'. Also, scientists for now at least must accept that 'people see genetic information as special' [7], even if in reality the information obtained by genetic and non-genetic tests can in some instances be very similar.

How Powerful Are the Genetic Tests That Currently Exist?

By the end of 2007, there had been over 200 genetic variations associated with performance and health-related fitness phenotypes [8] and that number continues to increase. In many cases, there has only been a single positive association with a relevant phenotype and consistent replication of the reported associations is obviously needed to increase confidence in the associations.

We are aware of just two rare mutations that each appears to have a powerful 'positive' influence on exercise-related phenotypes (although many rare mutations exist that have powerful 'negative' influences on exercise-related phenotypes). One rare mutation exists in the family of the Finnish cross-country skier Eero Antero Mäntyranta, where over 200 g of haemoglobin per litre of blood and extremely high haematocrit values >60% were reported [9]. EPO levels were normal but the mutation enhanced sensitivity to EPO. The responsible mutation in the EPO receptor gene was subsequently identified [10] as a premature stop codon that shortens the protein by 70 amino acids. A more active EPO receptor protein is the result, and is likely to have contributed to Mäntyranta winning three gold and four other medals at the Olympic games during the 1960s. Haematocrit in excess of 50% would attract investigation from the anti-doping authorities, although the World Anti-Doping Agency (WADA) 'Athlete Passport' concept combined with DNA analysis may allow an athlete competing today with such a mutation to demonstrate that erythropoiesis-stimulating agents had not been used.

A second naturally occurring rare mutation, this time with profound effects on muscle growth, has been observed in the myostatin gene (MSTN [11]). The authors reported the existence of a boy who was 'extraordinarily muscular, with protruding muscles in his thighs and upper arm'. The boy was homozygous for an extremely rare G to A intronic mutation in the myostatin gene and his muscle tissue showed no evidence of functional myostatin protein. This observation in a human parallels the deliberate knockout of the myostatin gene in mice, which has resulted in pronounced skeletal muscle fibre growth [12]. However, it is not certain that health status (e.g. cardiac function) will not be compromised by the absence of functional myostatin

protein, and there may be other detrimental changes such as changes in tendon properties [13]. Nevertheless, one possible kind of genetic test for physical performance could be to screen athletes for rare mutations such as those described in the EPOR and MSTN genes. The rarity and heterogeneity of such powerful mutations means that they would need to be identified using sequencing technology that allows base-by-base examination of relevant genes and gene portions, but even then would probably have a very low success or 'hit' rate. Nevertheless, future identification of an athlete with a particular rare, powerful mutation might help to explain the physiological capacity of that individual, provide clues regarding the type of events in which they may excel and even provide information that may be useful in the individualisation of his/her training.

In contrast to rare, powerful mutations, there are many common polymorphisms that are usually less powerfully associated with performance-related phenotypes. Here, we will consider as examples just the two potentially performance-related polymorphisms that have received the most research attention and one interesting polymorphism potentially related to injury risk.

As discussed in the chapter by Woods [pp. 72–87], a common insertion/deletion (I/D) polymorphism at the ACE locus is associated with circulating and tissue ACE activity, and associations of this ACE polymorphism with elite athletic status and exercise-related phenotypes (e.g. [14, 15]) have suggested the I-allele is associated with endurance phenotypes and the D-allele is associated with strength-related phenotypes. The polymorphism has also been associated with muscle fibre type proportion [16] which could go some way to explain these associations. However, other research groups have reported little or no association between the ACE I/D polymorphism and human physical performance (e.g. [17]). Our considered view is that the ACE I/D polymorphism is probably associated with some aspects of physical performance (e.g. physical capability at altitude) in some populations (e.g. well-trained Caucasians), but that the influence of this single polymorphism is probably modest. The technology required for screening athletes for the ACE I/D polymorphism (and most other common polymorphisms) is relatively commonplace (e.g. PCR and gel electrophoresis, or real-time PCR). However, the few percent (at best) of interindividual variability in a phenotype that may be attributable to ACE genotype is not sufficient for a genetic test at the ACE I/D locus to inform an individual (or his/her coach, for example) about future performance potential, which training mode may be most suitable, etc.

As discussed in the chapter by Yang et al. [pp. 88–101], a common polymorphism exists in the ACTN3 gene in which the element encoding arginine (R) may alternatively be a stop codon (X) that produces a truncated protein molecule with no known function. Studies of elite Australian [18] and Finnish [19] sprint/power athletes found none were of XX genotype. An ACTN3 knockout mouse displaying characteristics summarised as a fast-to-slow muscle phenotype change helps to explain mechanistic links between ACTN3 genotype and physical performance phenotype [20]. These

data suggest it is virtually impossible to become an elite sprint or power athlete without ACTN3, perhaps suggesting a powerful influence of the ACTN3 common polymorphism. However, in the general population it appears only ~2% of interindividual variability in muscle strength and sprinting speed are related to ACTN3 genotype [21, 22], and this seems somewhat at odds with the striking absence of the XX genotype in most elite sprint/power athletes. This paradox may be analogous with a classic study demonstrating that while maximal rate of oxygen uptake may have good statistical power in predicting endurance running performance amongst a wide range of running abilities, when a narrow (elite) range of performance is considered it is then running economy, a different phenotype, that becomes the dominant factor influencing performance [23]. Perhaps ACTN3 genotype (and thus the presence/absence of α-actinin-3 protein in muscle) is equivalent to running economy in that analogy, in that only amongst highly trained athletes does the practical significance of ACTN3 genotype increase to a meaningful extent. Nevertheless, even then, ACTN3 XX genotype does not seem to completely preclude success in power events [24]. The practical use of an ACTN3 genotype test to inform an individual (or his/her coach, for example) about future performance potential is thus still not clear, although of all common polymorphisms ACTN3 currently shows the most potential in this regard.

As discussed in the chapter by Collins and Raleigh [pp. 136–149], a GT dinucleotide repeat polymorphism in the tenascin-C (TNC) gene, which codes for an extracellular matrix protein, has been associated with the risk of Achilles tendon injury [25]. As in much research of genotype-phenotype interactions that is exploratory, it remains possible that the TNC polymorphism studied is not functional with respect to tendon injuries but merely in linkage disequilibrium with a different, functional polymorphism, perhaps within an adjacent gene. However, the logic for TNC and the protein for which it encodes being functional is strong. But regardless of the functionality, the potential exists to genotype athletes for the TNC polymorphism in an attempt to provide a basis for objective individualisation of training to minimise the risk of Achilles tendon injury or introduce other preventative measures. More research is needed to quantify the power of this polymorphism in predicting injury risk in specific sports, groups of individuals with specific geographic ancestry, etc., but the data are promising.

It is our view that while ACTN3 and TNC genotypes show promise, a large monogenic influence of a common polymorphism on physical performance or injury phenotypes is extremely unlikely. Future genetic testing of athletes will therefore need to account for a greater proportion of the genetic influence on interindividual variability via consideration of genetic variation at a number of loci simultaneously. The principle behind this polygenic approach has been demonstrated recently [26], although clearly more research is needed before the application of genetic algorithms to athletes can proceed with a valid scientific basis. Major decision-making regarding the careers or training/medical environment of athletes, based largely on the genetic testing of those athletes, is not scientifically justified until more research is conducted and therefore

would be unethical at the present time. Increased predictive power of genetic testing in athletes will be dependent upon the quality of the data that emerge from the future research [Trent and Yu, this vol., pp. 187–195] that will itself be dependent on the application of more advanced technology such as cheap, fast, whole-genome sequencing.

Who Should Be Able to Request Genetic Tests?

In the sport and exercise context, various people may request genetic tests for different reasons. With an accumulating number of available genetic tests, a wide range of genetic testing scenarios seems inevitable in the sport and exercise context. The five examples below show different individuals (athletes, parents, coaches, physicians) requesting genetic tests either for themselves or for others for a variety of purposes:

1 Genetic performance testing. Parents might request genetic tests for genes that determine adult peak maximal oxygen uptake for their child in order to decide whether to send their offspring to a special sports school.
2 Genetic testing for personalised training programmes. A coach might request genetic tests for trainability polymorphisms in order to decide whether weightlifters should take part in a high volume or high intensity strength training programme [for a more detailed debate, see 27].
3 Genetic sports disease testing. Sports associations and their physicians might use genetic tests to screen for variants associated with sudden death in order to identify individuals that are at high risk and to exclude such individuals from competing. A similar but non-genetic screening programme is performed in Italy [28] and has reduced sudden deaths but has also led to the exclusion of elite athletes from competitions.
4 Genetic sports injury testing. An individual could request a 'direct-to-consumer' genetic test [29] for tendon gene variants to predict the frequency of injury in order to decide whether to embark on a career as a professional football player.
5 Genetic insurance risk testing. An insurance company might request genetic tests for injury genes in order to determine insurance premium for a professional football player.

At the moment, most people would probably accept that adults should not be prohibited from requesting genetic tests for performance or sport and health related traits for themselves, in order to assist making life choices. We recommend, however, even in the case of 'mail order' or 'direct-to-consumer' tests [29] such as the commercially available ACTN3 R577X test [1] that these tests are accompanied by some genetic counselling. This could include counselling about potential unanticipated associations with disease, implications for relatives and other information which has been summarised in a review on 'ideal' genetic counselling [30]. The other recommendation is that genetic tests should, for the time being at least, only be allowed to be

requested by mature individuals (in the sense of mental capacity, in specific relation to the issue of genetic testing in sport) who understand the implications of a test.

Serious ethical concerns arise if genetic tests for an individual are requested by others. In the sport context this could be physicians, parents or coaches, and such tests could be used to discriminate in favour of or against athletes on the basis of performance-related or health-related genetic information. Some of these concerns are highlighted by a real case [31]. Eddy Curry is a professional NBA basketball player who missed games due to cardiovascular symptoms. His team (the Chicago Bulls) requested a genetic test for him based on the advice of a cardiologist. The athlete refused to do the test and was sold to the New York Knicks who did not request such a test. This is an example of an employer trying to force an employee to do a genetic test in order to then discriminate for or against the employee based on the test result. In this case, one can see the team's perspective as an employer that has a duty of care that might want to prepare itself for an acute cardiovascular problem, yet on the other hand one can understand the athlete's desire not to know or have others know about a serious genetic condition.

In an increasing number of countries, there is a legal framework that deals with genetic testing and resultant discrimination. In 2008, US Congress voted in favour of the 'Genetic Information Nondiscrimination Act' (GINA) to 'prohibit discrimination on the basis of genetic information with respect to health insurance and employment' in the USA [2]. In the sporting context, the WADA states in its 'Stockholm Declaration' [32]: 'The use of genetic information to select for or discriminate against athletes should be strongly discouraged', although the precise rationale for that statement is not given. It would be interesting to see whether the Eddy Curry case would be covered by GINA, although WADA also states that '[discouraging genetic discrimination] does not apply to legitimate medical screening'. However, both documents would prevent or strongly discourage professional football clubs, for example, from performing ACTN3 R577X tests on their players and using such information to discriminate against players. The international legal framework and related ethical issues for genetic testing have recently been reviewed [33].

The most serious ethical concern arises from the major fundamental difference between genetic and non-genetic testing which is that the DNA does not change throughout life and that therefore prenatal genetic tests could be performed to select an embryo with the 'best sport genotype' or to abort an embryo with the 'wrong sport genotype'. The legal prevention of such testing is important because there is evidence that some parents use pre-natal information to decide about whether or not to abort a pregnancy. In India and China, non-genetic ultrasound scans have been used to determine the sex of an embryo and this practice has greatly contributed to the estimated 80 million 'missing' females [34], a shocking statistic. Similarly, it may be that some genetic tests (not limited to genetic sex tests), including genetic tests of sporting potential, would be used by some parents during pre-implantation genetic diagnosis or to decide whether or not to abort an embryo.

Conclusions

The focus of this chapter has been on the potential use of genetic tests to predict performance and/or risk of exercise-related injury or illness. Various people may wish to conduct a sport-related genetic test on themselves, or on another person, for a variety of reasons. An individual may seek personal genetic information to assist him/her with their own sporting participation and career, by identifying the most suitable type of sport. A sports coach may wish to test the members of a youth team to assist in selection for a professional career or to individualise training. A physician may want to predict risk of injury or illness in an athlete and advise a coach regarding selection or preventative measures. An insurance company may seek to estimate risk of career-threatening injury or illness to an athlete based partly on genetic information. Despite the commercial availability of genetic tests today, the evidence available at present suggests that few, and probably none, of these or similar scenarios are scientifically justified – the genetic tests available at the moment are simply not powerful enough to provide valid data on which to base important decisions in sport. Furthermore, there are many challenging ethical issues regarding genetic testing of athletes. The imposition of genetic tests on individuals by third parties, and particularly the imposition of genetic tests on young people, is potentially susceptible to abuse. We propose that, for now at least, an individual should only be able to request a genetic test of their *own* DNA (i.e. not of another individual/employee/child), and only if he/she can demonstrate beforehand an understanding of the possible implications of the test result. We also recommend that an appropriate standard of genetic counselling is always provided. It is critical that these issues continue to be debated widely so that the tests already available, and the more powerful ones that are likely to emerge following improvements in knowledge and genetic technology, are only used in acceptable ways.

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The Future of Genetic Research in Exercise Science and Sports Medicine

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Abstract

Background/Aims: Genetic research is used to identify the relative contributions made by inherent abilities (nature) versus environmental effects (nurture) in human performance. The same approach allows a better understanding of how injuries or illnesses can result from sport or physical activity. Having identified the genes involved in athletic performance, there are the intriguing possibilities of using this information for talent search, developing individualized training programs and prevention of sports-related injuries. **Methods:** There are many interacting genes involved in athletic performance. This class of genes is often described as 'complex' and the mode of inheritance is called 'multifactorial'. Discovery of these genes is difficult using the conventional case control (association) studies. Recent genomic-based developments allowing high throughput SNP analysis are very promising. Potentially more exciting is the availability in the near future of cheaper and faster whole-genome sequencing technologies. **Results:** Genetic research in exercise science has produced a lot of data including the ability to draw a human exercise gene map. However, progress at the genetic level has been slow because gene-based association studies are not powerful enough to detect multiple small but cumulative gene effects. In future, the more efficient genomic-based research approaches will accelerate the finding of 'sports genes'. Data generated will be enormous, making it essential to have a direct link between the laboratory researcher and bioinformatics expertise. **Conclusion:** Genetics research has moved to the genomics era, i.e. the simultaneous testing of multiple genes is now possible.

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From Genetics to Genomics

The increasing demands required for success in sport at the elite level are usually approached through better talent search, more effective training regimens and improved infrastructural support. However, the developments achievable are usually incremental with the potential for quantum leaps only likely to come from cutting-edge research.

One major research interest is in the area of talent search. There are two main goals: (a) How can the most talented athletes be detected at an early age? (b) What

is the training potential for the individual, i.e. has the person with identified athletic ability reached his or her peak or is further improvement possible following training? Genetics-based research promises a lot in both of these quests.

Insight into how our genes work in the disease process provides a pathway to more effective treatments as well as earlier prevention strategies. Indeed, it is well accepted in clinical practice that knowledge of mutations in genes can be used to predict many years in advance, even before symptoms are apparent, that an individual will develop a disease. This is possible because a unique feature of a DNA (genetic) test comes from its *predictive* potential.

While inherently attractive, the applications of genetic knowledge in exercise science as well as the prevention of injuries in sports medicine remain elusive. Why? The genetics-in-disease paradigms are presently restricted to mendelian type genetic defects. These usually involve mutations in single genes having a major effect on protein output or structure and the result is a genetic disorder. Most mendelian type genetic disorders are relatively rare and their effects can be tracked through family pedigrees. In contrast, the more important public health problems in many communities, e.g. ageing, dementia, cardiovascular disease and most forms of cancer, are inherited in more subtle ways with the likelihood that there are cumulative effects from many genes contributing to the one disorder, and the effects of these genes can be modulated by interactions with the environment. In these so-called 'multifactorial genetic disorders', it is not possible to draw a pedigree and so trace the small but additive effects of multiple genes.

Normal traits, and included here would be the genes involved in human performance, fall into the class of multifactorial inheritance. The traditional genetic-based strategies to discover genes for multifactorial inheritance involve case-control studies (called association studies in genetics) that have many inherent difficulties including the high probability that false-positive or false-negative results will occur. There are a variety of reasons for failures in association studies including: (a) accuracy of the phenotype, (b) small numbers of subjects tested, (c) small numbers of gene markers. These will invariably affect the power in terms of study design [1]. (d) The candidate gene approach is itself a source of error with many unsuspected but important genes likely to be missed.

In contrast, the genomic-based whole-genome association studies allow multiple DNA markers (usually SNPs – single nucleotide polymorphisms) to be studied and these span the whole genome. Provided sufficient numbers of subjects are enrolled, it is highly likely that the whole genome association approach, because of the extensive coverage coming from the large number of SNPs used, will provide some important 'hits'. These will or will not confirm the earlier genetic association studies in sports research and will identify additional relevant loci and genes.

During 2006–2007, there was a significant increase in the number of genes for complex genetic traits discovered using the more sophisticated whole-genome association strategy. This was possible because of new developments in typing for DNA polymorphisms known as SNPs. Today, one can select a large number of SNPs based on the HapMap knowledge of linkage disequilibrium and type these DNA markers more

Table 1. Comparison of the traditional genetic association study and the genome-wide association

Feature	Existing genetic association	Genome-wide association
Genetic markers (SNPs)	Small number of SNPs – usually less than 20 and selected individually	Large number of SNPs, e.g. 500,000 – commercially available
Cases and controls	100–300 (individual research groups)	1,000–3,000 (these numbers possible with international consortia)
Population ethnicity and stratification	Self-reported and so lacking objective control (leading to the risk of population admixture)	Well controlled with a subset of analyzed SNPs (avoiding the population admixture issue)
Genes to be tested	One or a few candidate genes	All genes are tested without any assumed hypothesis
Effect sizes (odds ratio)	>5.0 (less likely to be true in complex trait)	1.5–2.0 (more likely to be identified in complex traits)

effectively across the whole of the human genome [1]. This approach has required the formation of international consortia to ensure that the numbers of subjects tested (in the thousands) are a magnitude higher than the more basic gene association studies (in the tens to hundreds of subjects; table 1).

Exercise Science

Talent Search

The many genes already implicated in human athletic ability have been captured in the form of a human gene map for performance and health-related fitness phenotypes [2]. The rapid developments in this area are illustrated by the 2006–2007 update which lists 214 autosomal gene sites and quantitative trait loci, an additional seven on the X chromosome and 18 mitochondrial genes. In contrast, the 2000 version of the map had only 29 identified loci. This catalogue is based on a review of the literature. Therefore, it will include many studies that remain to be confirmed.

The next level of sophistication for the above map would be verification of the various loci with a meta-analysis approach or more sophisticated strategies. The latter will come from whole genome association studies. Although still expensive (because of the large number of SNPs and samples used), the power generated is impressive, and so able to detect even small cumulative gene effects, i.e. quantitative trait loci. The attraction of the whole genome association approach will ensure that researchers pool their most important resource, i.e. DNA from elite athletes, by forming international consortia (table 1).

Individualized Training Programs

What is the best training program for a particular sport, or in the modern genetics jargon, can training programs be personalized based on the individual's intrinsic (i.e. genetic) strengths and weaknesses? Related to this is the dilemma facing national Sports Institutes when burnout occurs as a consequence of the training program. As well as being a personal loss to an individual who has dedicated a significant portion of his or her life to sport, burnout represents a significant financial loss to the sporting institutes.

This aspect of exercise science will be achievable in the longer term because the athletes can act as their own controls in comparative studies. However, it will continue to progress slowly until more is known about the genes and their interactions with other genes and the environment.

Sports Medicine

Preventing Sports-Related Medical Complications

Athletes will invariably suffer injuries as part of their training activities or during a sporting event. Most of these are assumed to be caused by the sport undertaken, but is this the complete explanation? The question now being asked is whether some individuals are more prone to injury because of their genetic makeup.

An example would be injuries to the Achilles tendon. Table 2 shows a number of factors that have been associated with Achilles tendon pathology [3]. A study in 2004 suggested that an individual with an Achilles tendon rupture had a significantly higher risk of getting a contralateral rupture as well [4]. To a geneticist, a bilateral problem suggests an underlying genetic factor is involved. Hence, not surprisingly, genetic studies have been undertaken to determine if Achilles tendon problems may also have an underlying genetic abnormality. The candidate gene chosen was *COL5A1* gene which is important for collagen formation. Mutations in this gene lead to a severe connective tissue disorder known as the Ehlers-Danlos syndrome. But would more subtle changes in this genes expression impair collagen and so predispose to problems with the Achilles tendon which is predominantly composed of collagen? The results showed changes at the 3′ end of the gene that appeared to correlate with the development of Achilles tendon pathology [3].

Like all genetic association studies, this is only a preliminary finding and would need verification, particularly in relation to functional studies. However, it illustrates the potential of this type of genetic research because confirmation of this genetic marker would enable the identification of those at greater risk for this sports-related injury, and so more effective prevention measures could be put in place to avoid it.

Sudden Cardiac Death during Exercise and Sport

A very tragic event is the sudden and unexpected death of a child or an otherwise healthy young adult during a sporting event, or while undertaking what appears to be routine

Table 2. Causes and contributing factors to Achilles tendon damage [3]

Intrinsic factors	Age, gender, body weight, vascular perfusion, nutrition, anatomical variants, joint laxity, muscular weakness or imbalance, the use of certain drugs, systemic disease and a history of previous injury.
Extrinsic factors	Type of activity, occupation, training, physical load, shoes, environmental conditions.

exercise such as jogging. Apart from the emotive issue, the death raises two important questions: (a) Could this have been prevented if the individual had been known to be at risk? (b) Is this a genetic problem, and if so are other members of the family at risk?

A number of important genetic disorders can first manifest during competitive sports, and unfortunately these presentations can be a sudden cardiac death. Examples include Marfan syndrome (e.g. dissection of the aorta) and familial hypertrophic cardiomyopathy (e.g. arrhythmia). Because of the predictive nature of DNA testing, it should be possible, in theory, to undertake the appropriate tests for the above two genetic disorders to determine if an individual is at risk. In practice though, the underlying mutations for these disorders are either family specific and/or involve multiple or large genes, and so the current genetic DNA testing approaches are not sophisticated enough to allow a definitive screening program to be instituted. However, this will change as genomic technologies evolve and allow multiple genes to be tested and/or DNA sequencing becomes cheaper and faster, allowing individual genes, no matter how large they are, to be sequenced (this approach is discussed at the end of this paper). In other words, the developments as we move from genetics to genomics will ensure that no matter how large the gene or how family specific is the mutation, it will be possible to screen individuals in a cost-effective way to identify potential mutations in all genes known to cause cardiovascular problems such as the ones described earlier.

With this technology and information available to both the individual and the sporting institute, will it be possible to prevent these tragic deaths? To answer this question will require more (some would say urgent) research. The challenges involved are many and are summarized in the editorial by Kjaer [5].

Ethical Social Legal Issues
There are a number of opportunities opened through predictive DNA testing in the sporting area. Nevertheless, the technology is still being developed that will allow better ways in which to use DNA tests. The ultimate of these would be a whole genome sequence – discussed towards the end of this chapter. In the meantime, some questions are worth considering. What duty of care is required of a sporting organization in terms of protecting its athletes? Presently, it would be reasonable to include as part of a fitness workup of new recruits that certain physical characteristics might be indicators of health risk during competitive sports, e.g. an individual who is very tall and/or has

features of Marfan syndrome. Individuals with these features should be referred for a formal clinical assessment. In this circumstance, the finding of an unexplained cardiac murmur or an aortic abnormality on imaging, confirms a high probability of Marfan syndrome and therefore significant risks associated with competitive sports.

On the other hand, whether a physical finding such as 'tallness' should automatically lead to a DNA test to screen for Marfan syndrome is being debated in terms of its feasibility and its cost versus benefit. Perhaps more problematic is what will the sporting organization do if it found one of its members had a mutation in the *FBN1* gene (the gene for Marfan syndrome) but no other clinical features? The DNA *predictive* test for Marfan syndrome is not a straightforward test because of the issue of variable severity, i.e. an *FBN1* gene mutation alone will not necessarily identify the severity of the disorder. What duty of care does the sporting body have to the athlete in this circumstance? Would it fail its duty of care if it allowed the individual to continue to train at the institute? On the other hand, would excluding the athlete because of the perceived health risk comprise unlawful discrimination?

Gene Doping

Invariably when the subject of genetics and sports comes up, the topic of conversation eventually shifts to gene doping. This is an unfortunate connection because the real challenges of genetics in sport, apart from the difficult research questions, focus around the use of genetic information and how this needs to be protected and used appropriately. Gene doping involves the manipulation of DNA, in particular gene transfer (also called gene therapy), to deliver a banned substance or to enhance or modify a natural gene product. Gene doping is no different to drug cheating or blood doping, and the fact that it involves DNA technology is incidental. In medical practice, gene transfer is still an experimental procedure with few successful results to date. In addition, the approach is risky and one of the complications already seen is interference with the function of a normal gene resulting in leukemia. Hence, gene doping is particularly inappropriate because it is banned by the World Anti-Doping Agency, and it involves an experimental procedure which in itself poses significant health risks to the individual [6]. What should not be forgotten in the emotive discussion around gene transfer and human performance is that in the longer term if gene transfer is able to be used effectively in the clinical setting, it might have an important application in treating some sport-related injuries [6].

Challenges for the Future

Frameworks for Research
All research conducted in human performance will go through the usual regulatory requirements, particularly assessment by a human research ethics committee,

to ensure that the appropriate safeguards are in place. Privacy issues are particularly sensitive when it comes to high-profile elite sportsmen and sportswomen because of the interest shown by the media in these individuals. In 2003, the United Nations Educational, Scientific and Cultural Organization adopted the International Declaration on Human Genetic Data [7]. This recognized that human genetic data have a *special status* because they:

- can be predictive of genetic predispositions involving individuals
- may have a significant impact on the family, including offspring, extending over generations and in some instances on the whole group to which the person concerned belongs
- may contain information the significance of which is not necessarily known at the time of the collection of biological samples, and
- may have cultural significance for persons or groups.

Not surprisingly, the potential misuse of genetic information in sport has attracted the attention of government, sports administrators and ethicists. One example is the extensive joint enquiry by the Australian Law Reform Commission and the National Health and Medical Research Council's Australian Health Ethics Committee into broad aspects of human genetics in Australia covering medical to non-medical uses of genetic information. The 2003 final report titled *'Essentially Yours: the protection of human genetic information in Australia'* [8] made two recommendations specifically directed to genetics and sport (see box).

Recommendation 38-1: The Australian Sports Commission should monitor the use of genetic testing and genetic information for identifying or selecting athletes with a view to developing policies and guidelines for sports organizations and athletes.
Recommendation 38-2: the Australian Sports Commission should develop policies and guidelines for sports organizations and athletes on the use of genetic information in relation to predisposition to sports-related illness or injury.

Direct-to-Consumer DNA Testing
A growth industry in the past few years has been direct-to-consumer (DTC) DNA testing. This describes the availability of DNA tests direct to the public without the involvement of health professionals. DTC DNA testing is offered for a number of 'products': (a) DNA genetic tests for medical conditions, (b) quasi-medical DNA tests such as dermatogenetics, i.e. based on an individual's own DNA information companies claim they can recommend appropriate creams or therapies that will ensure a youthful skin appearance. (c) Lifestyle DTC DNA tests – DNA markers are used for various nonmedical purposes such as genealogy searches or, in the context of sporting performance, the potential for an *individual* to excel in sport.

An example of a sports-related DNA test is the *ACTN3* gene test which is available through Genetic Technologies in Australia, and recently it has been licensed to a US-based DNA testing laboratory [9]. The scientific basis for linking *ATN3* to athletic performance was first established in 2003 and still remains the subject of interest and

research [10]. What remains to be determined is the benefit (if any) this DNA test has for predicting an *individual's* athletic ability, a question which is particularly relevant because the test is most likely to be used on a child.

Unfortunately, the rapid expansion in the number of DTC DNA testing laboratories, particularly those that are difficult to regulate because they are located offshore, and the broad claims made by these companies have the potential to hold back the more legitimate uses of DNA (genetic) testing. The negative effects on DNA testing by inappropriate use of DTC DNA testing, particularly when it comes to predictive and personalized genetics, is illustrated by a recent report from the US Government Accountability Office highlighting how four web-based nutrigenetics companies misled consumers [11].

New Technologies

The 'greatest transformational aspect of the Human Genome Project has been not the sequencing of the genome itself, but the resultant development of new technologies' [12]. The first human genome sequenced in 2003 is estimated to have cost about USD 3 billion. The next human genome sequenced in 2007 (that of Prof. James Watson) is said to have cost USD 2 million. Today, a human genome sequence can cost about USD 350,000 [13]. The next goal for a complete human gene sequence is the USD 1,000 price tag. Once this is achievable, the options for research studies will greatly expand with the sequencing of individuals providing a glut of comparative DNA data that will require sophisticated bioinformatics-based analysis.

New Paradigms

An important scientific fact emerging from the Human Genome Project is we have a simplistic understanding of what is a gene. This is well illustrated by the 2007 mapping of the pinot noir grape genome [14]. It showed this grape had slightly more genes than humans i.e. 30,000 versus 25,000. So what is it that makes humans more sophisticated than the pinot noir grape? 'Genes' per se cannot be the entire explanation, and so not surprisingly in terms of human performance, researchers will need to look beyond what is the traditional gene including:

Copy Number Variants. Although SNPs are the focus of current genetic research, there are other forms of variation within the genome, particularly the copy number variants. Gross structural changes leading to an increase or decrease in chromosome or chromosomal regions have been known for some time to produce genetic disease. However, much smaller copy number changes in the genome are also present with one estimate being that there are more than 1,400 copy number variable regions representing over 12% of the genome [14]. This remains an unexplored option in terms of how genes might influence human performance.

Epigenomics. Traditionally, the focus in genetic research is the gene and its DNA sequence. However, it is clear that gene expression can be altered without changing the actual sequence by chemically modifying the base cytosine or altering the proteins associated with DNA and chromosomes. These are known as epigenetic changes.

Today, there is evidence that sporadic tumors demonstrate epigenetic effects, i.e. either genes such as tumor suppressor genes are inappropriately turned off through epigenetic changes, or genes that should not be functioning like oncogenes, are inappropriately turned on because of loss of epigenetic control [14].

RNA is now seen as an important player in gene function. In particular, the potential for a new species of small (micro-) RNAs (miRNAs) to regulate DNA provides an additional epigenetic mechanism for gene regulation as well as protein diversity [14]. Do miRNAs explain why our genome in terms of gene number is similar to the pinot noir grape but the human proteome is considerably more complex? Time will tell, but the 2006 Nobel Prize in Physiology or Medicine for a species of miRNAs would suggest that research into sport will need to consider seriously the role played by epigenetics, and in particular miRNAs, in gene function.

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