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Genetics and sports

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Introduction: The limit of each individual to perform a given type of exercise depends on the nature of the task, and is influenced by a variety of factors, including psychology, environment and genetic make up. Genetics provide useful insights, as sport performances can be ultimately defined as a polygenic trait.

Sources of data: We searched PubMed using the terms 'sports' and 'genetics' over the period 1990 to present.

Areas of agreement: The physical performance phenotypes for which a genetic basis can be suspected include endurance capacity, muscle performance, physiological attitude to train and ability of tendons and ligaments to withstand injury. Genetic testing in sport would permit to identify individuals with optimal physiology and morphology, and also those with a greater capacity to respond/adapt to training and a lesser chance of suffering from injuries.

Areas of controversy: Ethical and practical caveats should be clearly emphasized. The translation of an advantageous genotype into a champion's phenotype is still influenced by environmental, psychological and sociological factors.

Emerging areas for developing research: The current scientific evidence on the relationship between genetics and sports look promising. There is a need for additional studies to determine whether genome-wide genotyping arrays would be really useful and cost-effective. Since exercise training regulates the expression of genes encoding various enzymes in muscle and other tissues, genetic research in sports will help clarify several aspects of human biology and physiology, such as RNA and protein level regulation under specific circumstances.

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Introduction

Darwin's theory of natural selection is that individuals with favourable traits are more likely to survive and reproduce than those without them. Those who are 'stronger' and who have adapted to the surroundings of their habitat are better equipped to handle the struggles they will face in their world. When we deal with natural selection, we often think of animals in the wild that adapt and change their traits to survive the wilderness. However, natural selection also applies to humans. Genetics is the science of heredity and variation in living organisms. It investigates gene function, genome structure, chromatin organization, recombination rate, mutation processes and evolutionary history, to provide a coherent understanding of the human genome and its complex relationship with human biology, physiology and disease.

For each individual, there is a limit to the capacity to perform exercise, which depends on the nature of the task and is also influenced by a variety of factors. The model for human physical activity patterns has been established not in gymnasia, athletic fields or exercise physiology laboratories, but by natural selection acting over millions of years of evolution. The adaptive pressures inherent in that environmental niche have exerted defining influence on human genetic makeup. Physical performance by vertebrates is hence regarded as constrained by trade-offs between antagonistic pairs of ecologically relevant traits, and between conflicting specialist and generalist pheno/genotypes.[1] Physical fitness has also a strong genetic component, up to 50%.[2] In an environment in which the selection criterion is combined with high performance across multiple tasks, increased performance in one function may impede performance in others. This hypothesis is supported by data from world-class decathletes, demonstrating that performance in the 100 m sprint, shot put, long jump and 110 m hurdles (which rely on explosive power and fast fatigue-susceptible muscle fibres) is negatively correlated with performance in the 1500 m race (which requires endurance and fatigue-resistant slow fibre activity).[1] Overall, these findings confirm the basic principle that athletes might be inherently predisposed towards specialist performance in one area (sprint/power) to the detriment of another (endurance). A challenging issue is the identification of the genetic background that influences the athlete's capability to excel in one sport discipline (i.e. sprint) rather than in a different one (i.e. marathon). Identifying the relevant genes to human athletic performance has been difficult, in part because each causal gene only makes a small contribution to overall heritability. In fact, the adoption of a 'single-gene-as-magic-bullet' philosophy is inconclusive and potentially misleading in the

athletic field, considering that the 2005 human gene map for physical performance and health-related phenotypes already includes 165 autosomal gene entries and quantitative trait loci (QTL), plus five others on the X chromosome. Additionally, 17 mitochondrial genes in which sequence variants have been shown to influence relevant fitness and performance phenotypes have been identified.[3,4] However, several genes among those listed are related to phenotypes such as total cholesterol and high-density lipoprotein cholesterol, and seem to have limited relationship to sporting performance. Moreover, many of the published genotype–phenotype associations have only been reported in people of one form of geographic ancestry. Therefore, it is not necessarily the case that such associations will exist in people with different geographic ancestry.

The physical performance phenotypes for which a genetic basis can be suspected include endurance capacity, muscle performance, determinants of tendon–ligament apparatus and physiological attitude to train. Consistent with the previous classification, the phenotypes of health-related fitness retained are grouped under additional categories, including haemodynamic (exercise heart rate, blood pressure and heart morphology), metabolism, anthropometry and body composition. This review will focus on the scientific evidence on the multi-faceted relationship between genetics and sport, illustrating the major genes associated with athletic performance (Table 1), reviewing the technical approach of genomic research in sports, highlighting advantages and ethical caveats of genotyping athletes.

Endurance ability

The increased ability to perform endurance exercise is strongly supported by enhanced mitochondrial function as suggested by increased mitochondrial gene expression, mitochondrial DNA and mitochondrial enzyme activity. Mitochondrial function is associated with aerobic physical fitness and insulin sensitivity, and may play an important role in the pathophysiology of type 2 diabetes. Peroxisome proliferator-activated receptor (PPAR)-delta (gene *PPARD*) and PPAR-gamma coactivator 1 alpha (gene *PPARGC1A*) are determinants of mitochondrial function in animals and *in vitro*. PPAR-delta, in particular, regulates expression of genes involved in lipid and carbohydrate metabolism, affects insulin sensitivity by modifying glucose uptake in skeletal muscle. A functional +294T/C polymorphism in this gene is also associated with predisposition to endurance performance.[5] The nuclear respiratory factors NRF1 and NRF2 coordinate the expression of nuclear and mitochondrial genes relevant to mitochondrial

Table 1: Major candidate genes associated with human athletic performances.

Endurance capacity
PPARD
Nuclear respiratory factors (NRF2)
PGC-1 alpha
HIF-1 alpha
EPAS-1 and HIF-2 alpha
Haemoglobin
Skeletal muscle glycogen synthase (GYS1)
ADRB2
CHRM2
VEGF
Muscle performance
CK-MM
ACTN3
MLCK
ACE
AMPD1
IGF-1
Tendon apparatus
ABO blood group
COL1A1 and COL5A1
TNC
Psychological aptitude
Serotonin transporter gene (5HTT)
BDNF
UCP2

biogenesis and respiration. Carriers of a polymorphism in the sequence of translation initiator ATG in the *NRF2* gene have higher training response in running economy than non-carriers, thus potentially explaining some of the inter-individual variance in endurance capacity.[6] The proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 alpha) is an important factor regulating the expression of genes for oxidative phosphorylation and ATP production in target tissues through coactivation of nuclear receptors. Muscle-specific expression of PGC-1 alpha improves the performance during voluntary as well as forced exercise challenges. Additionally, *PGC-1 alpha* transgenic mice exhibit an enhanced performance during a peak VO_2 exercise test, demonstrating an increased peak oxidative capacity, or whole-body oxygen uptake.[7]

The discovery of a family of proteins called hypoxia inducible factors (HIFs) has further contributed to enhance comprehension of the intricate mechanisms of response to hypoxia, as occurs in tissues that in some circumstances have to deal with increased oxygen demand, such as muscles working at high intensity. HIF-1 alpha is the primary transcriptional response factor for acclimation to hypoxic stress, which upregulates glycolysis and angiogenesis in response to low levels of

tissue oxygenation. Basically, the genes controlled by the HIFs include those coding for proteins that stimulate red cell production (mainly erythropoietin), as well as those encoding glycolytic enzymes, both pivotal in the attempt to achieve high levels of anaerobic performances, allowing a short-term increase in anaerobic power output when such an increase is needed and pO_2 is low. Removal of HIF-1 alpha causes an adaptive response in skeletal muscle akin to endurance training, and provides evidence for the suppression of mitochondrial biogenesis by HIF-1 alpha in normal tissue.[8] Since HIF-2 alpha, encoded by the endothelial PAS domain protein-1 (EPAS-1), is a sensor capable of integrating cardiovascular function, energetic demand, muscle activity and oxygen availability into physiological adaptation, DNA variants in *EPAS-1* influence the relative contribution of aerobic and anaerobic metabolism and hence the maximum sustainable metabolic power for a given event duration.[9]

The crucial role of haemoglobin in endurance performance has been well documented, in that an increase in its concentration in blood is associated with enhanced VO_{2max} and endurance capacity, which is also proportional to the increase in the oxygen carrying capacity of the blood. Subjects homozygous for intron 2, +16C/C or −551C/C in the haemoglobin gene have decreased oxygen cost of running, thus explaining part of the individual variation in the cardiorespiratory adaptation to endurance training.[10]

Genomic scans for glucose and insulin metabolism phenotypes in response to endurance exercise training yielded three QTL of interest. A promising locus for glucose effectiveness (an insulin-independent effect whereby glucose mediates its own disposal from plasma) influencing exercise training response was identified on 19q13 at the skeletal muscle glycogen synthase (*GYS1*) gene locus, which regulates glycogen storage in skeletal muscles. Two additional possible loci on 6p and 7q were captured for disposition index, which measures overall glucose homeostasis exercise training responses.[11] The adrenergic receptors are involved in several performance-related pathways and are therefore of particular interest as candidate genes for performance phenotypes. The β2-adrenergic receptor (*ADRB2*) gene, in particular, is a candidate for variation in endurance performance levels because of its contribution to the regulation of energy expenditure and lipid mobilization from human adipose tissue. The Arg16Gly polymorphism in this gene may be associated with endurance performance status in white men.[12] The acetylcholine receptor subtype M2 (*CHRM2*) plays a key role in the cardiac chronotropic response and DNA sequence variation at the *CHRM2* locus is a potential modifier of heart rate recovery in the sedentary state and after short-term endurance training in healthy individuals.[13] In its role as an endothelial cell proliferation and migration

factor, vascular endothelial growth factor (VEGF) can affect peripheral circulation. Therefore, individuals with at least one copy of the AAG or CGC promoter region haplotype have higher VO_{2max} before and after aerobic exercise training than subjects with only the AGG and/or CGG haplotype.[14] The mitochondrial uncoupling protein-2 (UCP2) negatively regulates reactive oxygen species generation. Recently, a linkage between *UCP2* gene and resting metabolic rate, but not with daily physical activity, has been demonstrated.[15]

Some other gene polymorphisms have been associated with sport performance, although results are still preliminary or controversial. These include polymorphisms in the alpha2a-adrenoceptor gene,[16] PPAR gene,[17] bradykinin beta 2 receptor and endothelial nitric oxide synthase 3 genes[18] and vitamin D receptor gene.[19]

Muscle performance

Heterogeneity is a property of all muscles and seems essential for their function. The combination of histochemical ATPase staining, antibody staining and electrophoresis has led to recognize that four major fibre types exist in mammalian skeletal muscles: one slow contracting form (fibre type I) and three fast contracting forms (fibre types IIA, IIB and IIX). Differences in the mechanical and energetic properties of isolated mammalian slow-twitch (ST) and fast-twitch (FT) muscles, motor units and muscle fibres have been well documented. In general, ST muscles are slower, less powerful and more economical at force generation than FT muscles. Furthermore, peak efficiency of ST muscle fibres occurs at slower shortening speeds than in FT fibres.[20] This heterogeneity covers all possible aspects of muscle contractile function, and is directed at optimizing the contractile responses and performing different motor tasks minimizing fatigue. The endurance capacity has been related to a predominance of ST fibres ($>50\%$), whereas FT fibres are related to power and speed capacity. Consistent with this evidence, power athletes and sprinters have a high proportion of FT muscle fibres with low-oxidative capacity compared with endurance athletes, who have a high percentage of ST muscle fibres.[21]

The creatine kinase isoenzyme MM (CK-MM) gene encodes the cytosolic muscle isoform of CK responsible for the rapid regeneration of ATP during intensive muscle contraction. In mice in which the CK-MM gene has been knocked out, lower fatigueability in skeletal muscle and cellular adaptations increasing aerobic capacity have been observed.[22] Under expression of this enzyme may therefore be responsible for muscular fatigue under normal circumstances, most likely because of the local cell compartment increase in inorganic phosphate

concentration. Furthermore, human studies of CK-MM gene sequence variation have shown a significant association between polymorphisms in this gene, increased cardiorespiratory endurance as indexed by maximal oxygen uptake following 20 weeks of training,[23] peak performance and less decline in force generation.[24] In particular, the A/G polymorphism in the 3′ untranslated region of CK-MM contributes to individual running economy responses to endurance training.[25]

The common genetic variation, which separates endurance athletes from sprinters, is probably due to natural selection. The actin-binding protein [alpha]-actinin-3 (ACTN3) is a highly conserved component of the contractile machinery in fast skeletal muscle fibres in mammals. This protein is only found in FT muscle fibres and it is responsible for the power necessary for successful sprinters or track cyclists. *ACTN3* is nearly always present among elite power athletes, whereas the R577X polymorphism (premature stop codon polymorphism) associated with complete ACTN3 deficiency is more prevalent among elite endurance athletes, such as marathon runners and rowers.[26,27] Detailed analysis of *ACTN3* knockout mouse muscle showed reduced fast fibre diameter, increased activity of multiple enzymes in the aerobic metabolic pathway, altered contractile properties and enhanced recovery from fatigue. This suggests a shift in the properties of fast fibres towards those characteristic of slow fibres, and provides a mechanistic explanation for the reported associations between R577X polymorphism, human athletic performance and muscle function.[28] Although the presence of ACTN3 has a globally beneficial effect on the function of skeletal muscle in generating forceful contractions at high velocity, the potential evolutionary advantage from the presence of ACTN3 related to increased sprint performance has been balanced by natural selection. Independent studies, however, failed to demonstrate a significant association between the R577X polymorphism and extreme endurance performance.[29,30]

Myosin light chain kinase (MLCK), a calcium-calmodulin-dependent multi-functional enzyme, plays a critical role in the regulation of smooth muscle contraction. Polymorphisms in this gene, especially the C37885A allele, are associated with post-exercise strength loss. Heterozygotes for this polymorphism also demonstrate greater strength loss compared with the homozygous wild type (CC).[31] Distinct beneficial effects on sprint and endurance athletic performance by different genotypes at a single locus have also been observed in studies of the gene encoding angiotensin-converting enzyme (ACE). The ACE gene has two alleles, termed 'I' and 'D'; the deletion (D) allele of the human *ACE* gene is associated with higher ACE activity than the insertion (I) allele in both tissue[32] and serum.[33] Although controversy exists, there are some evidences that genetic variation in the ACE gene might be

associated with many heritable traits, including physical, physiological, skill parameters and physical performance.[34] An increased frequency of the *ACE* I allele has been observed in elite endurance athletes.[35,36] Conversely, an increased frequency of the *ACE* D allele has been associated with elite sprint performance.[35,36] The mechanism underlying the association of the D allele with power-oriented, anaerobic sports is probably mediated through differences in skeletal muscle strength gain, since a greater training-related increase in quadriceps muscle strength has been associated with the D allele. Conversely, the I allele may influence endurance performance through improvements in substrate delivery and the efficiency of skeletal muscle, with subsequent conservation of energy stores.[34,36]

Adenosine monophosphate deaminase 1 (AMPD1) is a highly active enzyme in the skeletal muscle that plays an important role in the adenine nucleotide catabolism. Subjects with the TT genotype at the C34T *AMPD1* gene have diminished exercise capacity and cardiorespiratory responses to exercise in the sedentary state.[37] Moreover, carriers of the T allele have a limited training response of ventilatory phenotypes during maximal exercise[37] and a reduced submaximal aerobic capacity.[22] The insulin-like growth factor 1 protein (IGF-1) increases muscle mass and possibly strength. Accordingly, carriers of the 192 allele of the *IGF-1* promoter microsatellite are characterized by greater quadriceps-muscle strength gains compared with non-carriers.[38] However, this study included older adults (aged 52–81), who have different metabolic characteristics, especially in terms of growth factor levels, cytokines, prior habitual activity and so on than young aspiring athletes. Therefore, this study does not provide good evidence that variation at this locus influences likelihood of success in sport.

Tendon apparatus

Painful tendon disorders are a major problem in competitive and recreational sports[39,40] and in the workplace.[41–48] The essence of tendinopathy is a failed healing response, with haphazard proliferation of tenocytes, some evidence of degeneration in tendon cells and disruption of collagen fibres and subsequent increase in a non-collagenous matrix.[43,49] The aetiopathogenesis of tendinopathy and the underlying molecular changes are still ill defined. Although repetitive forces have often been implicated in the pathogenesis of tendinopathy, recently, differential strain, 'stress shielding' and even under-use have been put forward as alternative biomechanical explanations to the classical overuse model for the pathogenesis of tendinopathy.[50]

An interaction between the various intrinsic and extrinsic factors with the genetic make up of a given individual might increase the likelihood of that individual developing tendinopathy. However, investigations into the genetic factors involved in the aetiology of tendinopathy are still in their infancy. An underlying genetic factor as a contributing cause to tendon injury was originally proposed because of an association between the ABO blood group and the incidence of Achilles tendon ruptures or chronic Achilles tendinopathy evident in Hungarian and Finnish populations with blood group O.[51] These studies implied that ABO or closely linked genes on the tip of the long arm of chromosome 9 could be associated with tendinopathy or tendon injuries. The gene for ABO on chromosome 9q34 encode for transferases which, apart from determining the structure of glycoprotein antigens on red blood cells, may also determine the structure of some proteins of the extracellular matrix of tendons.[51] However, this association between the proportions of ABO blood groups and Achilles tendon rupture was not evident in Scotland.[52] The findings from studies performed in different geographic areas could result from peculiarities in the distribution of the ABO groups in genetically segregated populations.[52]

A genetic component has been implicated in tendinopathies involving the Achilles tendon[53,54] and the rotator cuff tendons.[55] Polymorphisms within the *COL5A1* and tenascin-C (*TNC*) genes have been associated with Achilles tendon injuries in a physically active population[53,54] and the rotator cuff tendons.[55] Polymorphisms within the *TNC* and *COL5A1* genes have been associated with Achilles tendon injuries in a physically active population.[53,54] The association of polymorphisms within the *COL5A1* and *TNC* genes with rotator cuff injuries has not been investigated to date. *COL5A1* gene has a role in the pathogenesis of Achilles tendinopathy and it has been observed that South African individuals with the A2 allele of this gene are less likely to develop Achilles tendinopathy.[54] Although no direct link with *COL5A1* gene has been demonstrated, the genes encoding for collagen I and III, namely *COL1A1* and *COL3A1*, show relatively high but variable levels of expression in normal tendon, and significantly increased expression of both genes is found in painful tendinopathy.[56] Allele distribution of the Guanine–Thymine (GT) dinucleotide repeat polymorphism in the *TNC* gene is associated with Achilles tendon injury.[53] Alleles containing 12 and 14 GT repeats were significantly higher in patients with Achilles tendon injuries, while alleles containing 13 and 17 GT repeats were higher in the asymptomatic controls.[53] A possible biological explanation for the involvement of TNC in the aetiology of Achilles tendon injuries could be explained by abnormal mechanical loading leading to altered synthesis of TNC,[57] which could disrupt the

regulation of cell matrix interactions in the tendon,[58] with onset of apoptotic changes in the tenocytes.[59] The exact role of *COL5A1* and *TNC* genes in the pathogenesis of tendinopathy is still debated, and the current evidence does not allow to clarify whether or not *COL5A1* and *TNC* genes are the ideal markers of tendinopathy.[54] On the basis of current evidence it is difficult to conceive that only a single gene and not multiple genes are involved in the pathogenesis of tendinopathy. Thus, additional investigations need to be performed to identify these genes.

Ligamentous apparatus

Failure of ligamentous apparatus is challenging, especially when it occurs in athletes.[60] A genetic component has been hypothesized also for damage to the ligamentous apparatus. A family history of shoulder instability in first-degree relatives was found in 24 of 100 patients who had been operated on for recurrent anterior shoulder instability.[61] In a case control study of 171 surgical cases and 171 matched controls, a familial predisposition towards tearing the anterior cruciate ligament of the knee was found.[62] The risk of cruciate ligament ruptures and shoulder dislocations has been associated with a polymorphism in the *COLIA1* gene.[63]

Genetic study in ligamentous apparatus are still at their infancy. An involvement of the same genes involved in tendinopathy has been proposed.[64] However, tendon and ligaments have different function, and these hypotheses still require to be confirmed.

Potential for gene therapy

Ligaments and tendons have a relatively similar structure, but different functions. They have dense, collagenous structures with few cells and both can heal after injury, but the repaired tissue is weaker than normal and liable to rerupture.[65] In patients undergoing surgical repair, the formation of post-operative adhesions is a common complication.[65] Ligaments and tendons fail to heal spontaneously and are a major clinical problem.[65]

Sustained gene expression lasts for about 6 weeks in tendons, possibly long enough for clinical applications.[66,67] Healing tendon has proportionately higher levels of type V collagen and persistently elevated levels are present up to 52 week after injury in the rabbit medial collateral ligament.[68] Elevated levels of collagen type V may favour the formation of smaller type I collagen fibrils, which results in reduced

mechanical strength.[68] Transfection of human patellar tenocytes with specific antisense oligonucleotides demonstrated a reduced amount of collagen type V.[69]

Most gene therapy approaches focused on the delivery of BMP-12 (GDF-7) and BMP-14 (GDF-5, CDMP-1) because of their effects in promoting tenogenesis and ligamentogenesis.[65] Animal studies using adenovirus vectors[70–73] or platelet-derived growth factor cDNA[74] to enhance repair obtained promising results.

Dai *et al.*[75] investigated the transfection of an adenovirus containing the reporter gene LacZ, in primary-cultured human rotator cuff tendon cells and in a rat Achilles tendon healing model *in vivo*. They found that adenovirus can be used to deliver a gene of interest to cultured human rotator cuff tendon cells and healing tendon, with gelatin sponge implantation enhancing adenoviral transfection efficiency *in vivo*. A variation in this approach has been studied *in vitro* to deliver TGF-β1 and IGF-1[76] to repair cells migrating from injured anterior cruciate ligament.

Psychological aptitude

Human physiological trait variance has both an environmental and genetic basis, although the classic gene–environment dichotomy is clearly too simplistic to understand the full range of variation for most proximate determinants of athletic performance.[77] Although it is undeniable that success in competition has a strong psychological background, represented by mental toughness, tactical astuteness and motivation to endure pain during training and competition, the association between genetics and sport psychology is still largely underinvestigated. Some earlier studies have indicated that genetic factors account for ~29–62% of the variance in daily exercise behaviour and 35–83% of the variance in sports participation.[15,78] Hence, despite the clear heritability of exercise behaviour, work on the human genome has only recently implicated specific genes that are related to the motivation to exercise or to the maintenance of exercise behaviour.

There is evidence that polymorphisms in 5′-flanking regulatory regions of serotonin transporter gene (*5HTT*) encoding code for long (L) or short (S) alleles might be associated with human adaptive ability to control emotions, thus representing an intriguing area of investigation in athletes, who are subjected to high emotional pressure on a regular basis. Psychological testing of aggression in female athletes showed that scores of indirect hostility were higher, but scores of irritability and negativism were lower in female athletes with the SS genotype as compared those with LS and LL genotypes.[79] Brain-derived

neurotrophic factor (BDNF) is a peptide growth factor that has broad influence on central and sensory neuronal function, on development of the vasculature and on neuronal growth and regeneration in the hippocampus as well as in other brain regions, in the spinal cord and in skeletal muscle. Preliminary results suggest that polymorphisms in the *BDNF* gene, especially the G/A substitution at nucleotide 196, may exert a small, direct influence on positive mood but strong effects on ratings of perceived exertion and heart rate in response to a bout of aerobic activity.[78]

Technical issues

Given the huge amount of available scientific information, the relative importance of factors over which an athlete has little or no control, such as genes, compared with those that can be modified, such as environment, training regimens and nutritional supplements needs scrutiny. One key way to investigate the strength of the relationship between genetics and sports performance is to conduct twin/family studies, which would provide an estimate of the inter-individual variability that is inherited. Unfortunately, there are no such studies published so far, other than those analysing walking ability in relatively healthy older women[80] or sports participation in adolescence.[81]

Therefore, two potential approaches can be advocated to investigate the relationship between genetics and sports. Genetic association studies offer a potentially powerful approach for mapping causal genes with modest effects, but are limited because only a small number of genes can be studied at a time. In contrast, genome-wide association (GWA) studies will soon open new frontiers in our understanding of human biology, physiology and pathology.[82] Advances in genetic knowledge, high-throughput genotyping technologies, statistical analysis algorithms and a flood of data on human genetic variation from the Human Genome and HapMap projects have made GWA studies technically feasible, allowing to identify genetic variants that are associated with complex human diseases.[83] DNA microarray, or DNA chips, is fabricated by high-speed robotics, generally on glass but sometimes on nylon substrates, for which probes with known identity are used to determine complementary binding, thus allowing massively parallel gene expression and gene discovery studies. An experiment with a single DNA chip can provide researchers information on thousands of genes simultaneously—a dramatic increase in throughput. Qualitative or quantitative measurements with DNA microarrays use the selective nature of DNA–DNA or DNA–RNA hybridization under high-stringency conditions and fluorophore-based detection. DNA arrays are

commonly used for expression profiling, i.e. monitoring expression levels of thousands of genes simultaneously, or for comparative genomic hybridization. With the skill and all the necessary tools in hand, the potential to extend this powerful approach to investigate athletic performance is unquestionable, in that it will allow a wide exploration of the genetic basis in elite athletic performance.[84] Since GWA studies mostly rely on commercial single-nucleotide polymorphism (SNP) chips, for which a common evaluation criterion is global coverage of the genome,[85] one of the major obstacles originally encountered in this approach was the identification and mapping of numerous polymorphisms that could easily be and inexpensively typed. However, this challenge was overcome with the sequencing of the human genome and the subsequent cataloguing of SNPs. In most cases, however, it remains prohibitively expensive to genotype all the desired samples using a genome-wide genotyping array, so multi-stage designs are an attractive cost-saving alternative.[83] The commercial availability of mass-throughput oligonucleotide array-based genotyping platforms at affordable prices would hence make genome association scans a reality, and bring us closer than ever to elucidating the genetic mechanisms of sport performance.[86] Some examples are already available, such as the identification of QTL on chromosome 11 that harbours genes influencing resting heart rate variation at baseline and in response to regular exercise training,[11] exercise stroke volume, cardiac output[87] and endurance exercise training responses in insulin action and glucose metabolism phenotypes on chromosome 19q as well as 6p and 7q.[11] With the advent of high-throughput microarray-based epigenetic technology (e.g. ChIP-on-chip and ChIP-seq), genetic testing will go beyond assessing gene expression, to explore gene regulation activity. It will also overcome traditional and labour-intensive protocols, such as restriction fragment length polymorphism–PCR, allele-specific oligonucleotide hybridization, non-PCR oligonucleotide cleavage technology and real-time PCR, providing the simultaneous measurement of the relative expression levels of thousands of individual genes.[88] Although financial costs are currently a major obstacle, the future prospects are good.

Gene doping: the next threat

According to the creed of the Modern Olympics, 'the most important thing is not to win but to take part, just as the most important thing in life is not the triumph but the struggle'. Regardless of this unquestionable ethical principle, we all know that the creed of most professional athletes is instead 'winning isn't everything; it's the only thing'. As

many more individuals of each species are born than can possibly survive, there is a frequently recurring struggle for existence. If competition lies at the very heart of evolution, the fact that there are top-class, professional athletes who embrace any type of aid to win on the athletic field should not be really surprising. Doping is conventionally regarded as the unethical use of performance-enhancing substances or methods, which targets bodily functions including cerebral, metabolic, cardiovascular, respiratory, haematological and, in the very near future, genetic.[89] Together with the rapidly increasing knowledge on genetic therapies as a promising new branch of regular medicine, the issue has arisen whether genetics and, especially, gene therapy might be abused in the field of sports. Gene or cell doping is defined by the World Anti-Doping Agency (WADA) as 'the non-therapeutic use of genes, genetic elements and/or cells that have the capacity to enhance athletic performance'.[90] Gene therapies stimulating erythropoiesis and improving the aerobic performances, along with transfection of genes supposed to be used in patients with degenerative muscle diseases and capable to enhance the muscular output are supposed to be attractive alternatives for traditional performance-enhancing drugs, like human recombinant erythropoietin and anabolic agents. Myostatin, a member of the transforming growth factor-beta family of proteins that plays a fundamental role in regulating skeletal muscle growth during embryogenesis, is a paradigmatic example. Since studies in both humans and animals discovered that decreasing the levels of this growth factor or inhibiting its function can dramatically increase muscle size, and a number of therapeutic applications of myostatin inhibition to the treatment of myopathies and muscle atrophy have been proposed,[91] there is a growing concern that myostatin inhibitors may be among the next generation of ergogenic pharmaceuticals or even in the vanguard of 'gene doping' technology.[91]

Several issues predict success for this new form of cheating. Transfection of genes virtually identical to those naturally represented in the human genome should outweigh the problem of positive anti-doping testing, making gene doping almost undetectable by traditional laboratory techniques.[92] Then, as some forms of gene doping are expected to exhibit long-lasting or perpetual effects (i.e. transfecting erythropoiesis stimulating substances), abolishing the need for repeated administrations of exogenous pharmacological agents, athletes might consider it a more attractive option to traditional doping.

Genetic testing might be helpful also in the anti-doping context. First, establishing a basic genetic profile in the young athlete would prevent the uneventful possibility of gene transfection, since each deviation from an inherited pattern would be unquestionably interpreted as pathology or unfairness. Furthermore, the identification of

polymorphisms associated with variability in metabolism of hormones and proteins would enhance the diagnostic efficiency of traditional anti-doping tests, which are known to be unreliable under some circumstances. Testosterone abuse is conventionally assessed by the urinary testosterone/epitestosterone (T/E) ratio, levels above 4.0 being considered suspicious. The large variation in testosterone glucuronide excretion and its strong association with a deletion polymorphism in the *UGT2B17* gene challenge the accuracy of the T/E ratio test.[93] This is a valuable example on how consideration of the genetic variation in disposition of androgens will improve the sensitivity and specificity of the testosterone doping test. This is of interest not only for contrasting androgen doping in sports, but also for detecting and preventing androgen abuse in the society.[94] However, gene transfection in muscle tissue would probably only be detectable from sampling of muscle tissue, since urine and blood may show no detectable evidence. There might also be additional challenging factors such as microchimerism, meaning that difference from the expected individual genomic signature is possible.

Conclusions

The human race has celebrated its athletes since the ancient Greece. But from where does all this admiration for feats of incalculable skill and ability (and subsequent disillusionment and anger when those feats prove in doubt) derive? The historical debate on the relative influences of natural selection (genes) and environment conditions on human athletic performances has been characterized by extreme positions, leading to reductionist and polemic conclusions.[95] However, human physical capability is influenced by many genetic factors, and physical capability phenotypes are highly polygenic. Recently, the development of technology for rapid DNA sequencing and genotyping has allowed the identification of some of the individual genetic variations that contribute to athletic performance. Using probability calculations, the real chance of an athlete displaying a perfect arrangement of all the acknowledged 'positive' polymorphisms influencing athletic capabilities is low. Moreover, as the discovery of variants associated with human sport performances steadily increases, such probability will become statistically negligible.[96] Therefore, one would be tempted to conclude that genetic testing in sports might lead to much wasted efforts. However, this is mostly an ethical than a practical issue. First, elite athletes do not generally exhibit marked differences across a number of polymorphisms from the general population, but they might simply carry more of the positive alleles than the general population, and they could

be hypothetically identified using that knowledge. Then, genetic studies have been accomplished until recently by studying one or a few genes at a time. With the advent of microarrays, expression analysis has taken giant leaps forward with the capability to screen in the range of several thousands of genes simultaneously (up to 1 000 000 different probes per cm^2).[97] However, with the amounts of sequencing data and expression profiling data being generated, a genome-wide screen developed on the basis of simple epidemiological associations would be meaningless or even misleading, unless (i) the gene–environment interaction is comprehensively understood, (ii) the potential gene–gene synergies are recognized, (iii) the exercise-induced regulation of genes encoding proteins supporting athletic performances, such as proteins involved in fatty acid recruitment and oxidation, is identified[98] and (iv) powerful bioinformatics technologies are available to statistically analyse the data. Statistical methods for joint linkage and linkage-disequilibrium mapping strategy are especially needed to resolve strong multi-locus association signals and dissect the genetic architecture of complex traits, such as sport performance.

Although we all would agree that genetic testing is not aimed to replace the findings on the athletic field, under the most appropriate circumstances it might be less invasive, less expensive and more accurate than conventional *in vivo* or *in vitro* analyses. Moreover, although it should be discretionary and regulated on athletes,[99] it represents a great opportunity to build a solid bridge towards a rational and personalized training framework, one of the future challenges of physiology and sports medicine.[3] Although genotyping of athletes will become an option when performance or injury genes have been definitely identified, many may still regard the practice as unethical. There are two extreme positions when considering this issue. The former is consistent with the thesis that 'if you want your children to become great athletes, just marry a great athlete'. In fact, a deep knowledge of the inherited basis of athletic performances might induce to plan offsprings between individuals carrying favourable genotypes to generate who is expected to be the champion of the future. Furthermore, if a young athlete is aware of lacking genetic potential for a certain sport, will he or she be less likely to participate, even not expecting to become a famous, top class athlete? Therefore, the effect of genetic testing on sport participation and the social development of children should be considered in great depth, even though the possibility could be raised that guiding children towards the most suited athletic discipline may increase participation rates as positive feedback is obtained. On the other hand, nowadays genotyping can not be really considered different from saying to a very short person that he/she will not be chosen for the national basketball team because of the height. The

early identification of young athlete's predisposition for a certain type of sport might be a vital component of many sport programmes and would also be useful to guide children towards the most suited athletic discipline. This is especially true in competitive sports, where even slight differences in athletic skill can separate the winner from the rest of the competitors. Once more, however, the most rationale approach probably lies in the middle. Several gene polymorphisms might strongly predict the predisposition to becoming a top-class athlete, but an advantageous genotype not always translates into the phenotype of a champion, since a variety of psychological and environmental factors still influences gene expression. Sport performances are also the result of hours spent in focused, prolonged, intensive training, and a favourable genotype is not be enough to produce a champion. Although much work needs to be done to establish influence and interaction of genes across a range of athletic parameters, genetic testing will indeed help identify individuals with advantageous physiology, morphology and maybe psychology, those with a greater capacity to respond/adapt to training and those with a lower chance of suffering from injuries. Contextually, since exercise training regulates the expression of genes encoding various enzymes in muscle and other tissues, genetic research in sports will help clarify several aspects of human biology and physiology, such as RNA and protein level regulation under specific circumstances.[100] Accordingly, the British Association of Sport and Exercise Sciences (BASES) Molecular Exercise Physiology Interest Group has recently produced a position stand to advise on current issues in genetic research and testing in sport and exercise science (BASES position stand on Genetic Research and Testing in Sport and Exercise Science). This statement clearly highlights that genetic testing (i) might be useful for the development of genetic performance tests, (ii) may also be applied for pre-participation risk screening and may prevent sudden deaths during sport, (iii) might in future also be used to identify those who are most likely to benefit medically from exercise programmes and (iv) may become more important in anti-doping activities where it could be used for identification purposes (genetic fingerprinting) and more direct antidoping testing.

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