MOLECULAR-GENETIC PREDICTIONS IN SELECTION OF SPORT TALENTS AND ETHICAL ASPECT OF THEIR APPLICATION

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(Review paper)

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Abstract
The genetic basis of complex athletic phenotypes is still poorly understood and difficult to study. The molecular basis of genetic variation related to sport performances resides in both nuclear (nDNA) and mitochondrial DNA (mtDNA). Variabilities in skeletal lengths, skeletal breadths, limb circumferences, and bone mass is genetically determined. In nDNA, relevant gene variants include the angiotensin converting enzyme (ACE), actin 3 (ACTN3), myostatin (MSTN), insulin-like growth factor I (IGF-1), phosphoenolpyruvate carboxykinases (PEPCK) and erythropoietin (EPO), among others. Genetic factors account for ~40–60% of the variation in aerobic performance and cardiac function, 50–90% of the variation in anaerobic performance, 30–70% of the variation in muscular fitness, and 20–30% of the variation in muscle dimensions. Adaptive polymorphisms in mtDNA may directly affect maximum performance capacity. Four obligations of professional ethics for the researcher interacting with the application of molecular tools are: autonomy, beneficence, non-maleficence, and justice. Talent selection is difficult in children although it could guide early exposure to sport-specific training. Recent advances in scientific knowledge and the availability of modern technology could provide an opportunity for the direct genetic selection of athletic talent. Talent selection and selection is institutionalized for many sports around the world. Although unique combinations of genetic and environmental factors result in elite-level performance, these factors are complicated and generally unpredictable. The question is whether genetic screening techniques are able to identify an innate advantage as part of talent identification programs and is the focus of this review.

Key words: genetics, sport, talent, ethics

Introduction
Talent is exceptional performance that is partly innate, relatively domain-specific, found only in a limited number of individuals, and partly identifiable at an early stage of development Falk et al. (2004) emphasised the importance of early talent detection and described three aspects of talent identification (TID) - physiological, psychological and sociological. Early TID methods are designed to identify those children with favourable physiological traits suited for a particular sport based mainly on genetics.

Early TID and selection is institutionalized for many sports around the world. TID and development has become a vital component of many sport programs. This is particularly true in Australia where significant resources have gone into developing a national Talent Search program that is implemented through the Australian Institute of Sport. The significance of TID in this country probably stems from greater competition between sports for talented individuals (Hoare and Warr, 2000) within a relatively small population base compared to the sporting superpowers.
The literature reviewed indicates a high success rate of TID within sports that are individual and repetitive with specific anthropometric and physiological requirements. However, the use of TID within sports requiring more decision-making and "game sense" requires further investigation before accurate prediction models can be accepted (Lidor et al. 2005). However, can the same TID principles be applied to team sports? Not surprisingly, some research (Reilly and Gilbourne 2003; Pienaar et al. 1998) showed that the use of genetically determined qualities did not play a major role in the early detection of elite performers. Other factors contributing to success in these sports included game knowledge and game sense, team coherence, maturity, anticipation and decision making.

The emphasis on screening for structural characteristics in these individual sports is explained by Patel and Greydanus (2002) who cite the following characteristics as having a large genetic influence: height, arm length, muscle size, strength and muscle fibre composition, heart size, resting heart rate, lung size and volume and, flexibility of joints. When considering the use of gene-based technologies for TID, one must consider the degree to which a physical ability is determined by genetic make-up. Aerobic performance and VO$_2$max commonly appears in the literature as factors that are largely determined by genetics. Namely, 30-70% of an individual’s cardiac structures and response to cardiopulmonary exercise is genetically predetermined. MacArthur and North (2005) stated that genes account for more than 40% of the variance in oxygen uptake at the ventilatory threshold. Hohmann and Seidel (2003) explained that the percentage of VO$_2$ max attributed to genetics has shifted from 90% in the 1970’s to approximately 50% currently.

Training is also critical for success, but it is recognized that it is only one component leading to the status of an elite athlete. Genetic screening is also being used to individualize training programs to suit a person’s genotype. Still, our understanding of the effects of human genomic variation on the ability of tissues and organs to be trained remains limited.

Knowledge concerning the relationship of genetics to human performance has grown markedly during the last quarter century. The human genome consists of ~3.4 billion base pairs of DNA and has ~50,000 active genes. All of these genes are likely polymorphic, except for those sequences whose polymorphism would result in phenotypes incompatible with life.

One of the last human gene maps for physical performance and health-related phenotypes included 140 autosomal gene entries and quantitative trait loci, plus four on the X chromosome (Fig. 1) (Wolfarth et al., 2005). Such variables in the general population are mostly related to quantitative, multifactorial phenotypes that are influenced by multiple genes (polygenic) and environmental factors.

![Fig. 1 Human performance and health-related fitness gene map. The map includes all gene entries and QTL that have shown associations or linkages with exercise-related phenotypes. The chromosomes and their regions are from the Gene Map of the Human Genome web site hosted by the National Center for Biotechnology Information, National Institutes of Health](image-url)
Mitochondrial DNA (mtDNA) is a 16,569 nucleotide, closed-circular molecule, located within the mitochondrial matrix, and present in thousands of copies per cell. There are 16 mitochondrial genes in which sequence variants have been shown to influence relevant fitness and performance phenotypes (Fig. 2) (Walace 2005). The mutation rate of mtDNA is higher than in nuclear DNA. It is now clear that not all mtDNA variation is deleterious. For example, parents might consider changing the mtDNAs of their children in hopes of increasing their potential for athletic performance. (Scott et al., 2009).

Figure 2. Polymorphisms associated with sport performances: MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy and ragged red fiber disease; NARP, neurogenic muscle weakness, ataxia, retinitis pigmentosum; LHON dystonia. (Wallace, 2005)

Examples For Gene Association With Sport Performance

Peptidyl dipeptidase angiotensin-converting enzyme (ACE) is a key enzymatic component of the rennin-angiotenzine system, having critical roles in the regulation of blood pressure and salt/water balance influencing endurance. The ACE gene shows a polymorphic insertion of a repetitive element in intron 16. This 287-bp insertion/deletion (I/D) polymorphism has been directly linked to inter-individual variation in plasma and tissue ACE activity. The ACE I/D polymorphism was the first specific gene variant to be associated with human physical performance (Montgomery et al., 1998). Since that time, the ACE gene has become, by far, the best-studied locus in this regard. The I/D polymorphism is associated with differences in response to training, including muscle endurance, and anabolic strength improvements. It has also been associated with the proportion of type 1 fibers in muscle, and cardiac muscle growth. The associations of ACE polymorphisms with fitness, and elite sports performance remain more controversial.

A study for maximum duration of standardized repetitive elbow flexion with a 15-kg barbell was done. After 10 weeks of military training, the duration of exercise was significantly prolonged in the 66 individuals of II or ID genotype, but not for the 12 DD homozygote. Thus, II homozygote showed an 11-fold greater improvement when compared to those with the DD genotype (Montgomery et al., 1998).

Another study of 47 individuals demonstrated that ACE II genotype carriers had a 6.3 ml/kg/min higher VO\textsubscript{2max}, than the ACE DD group and a 3.3 ml/kg/min higher VO\textsubscript{2max} than the ACE ID genotype group (Hagberg et al., 1998). However, the validity of any association between ACE genotype and VO\textsubscript{2max} remains unproven.

The I-allele is also associated with a success in rapid ascent to high altitude and success in ascending beyond 8,000 m (Tsianos et al., 2005). Such effects may be dependent upon I-allele associated gains in VO\textsubscript{2max}, or in metabolic efficiency (Bischoff et al., 2003). In general, the I-allele has been associated with elite athletic performance in endurance-orientated events (Nazarov et al., 2001), and the D allele with strength/power-orientated performance (Myerson et al., 1999).

A functional variant in Actin 3 (ACTN3) is believed to impact acceleration performance (Yang et al., 2009) via its role in skeletal muscle. The variant R577X, dbSNP rs1815739 induces a premature stop to the alpha-actinin-3 protein at amino acid 577 (which is usually an arginine). Individuals homozygous for the stop codon completely lack the ACTN3 protein, although this deficiency is not associated with any known disease. This coding sequence variation was first identified by North and colleagues (1999). Walsh et al., (2008) first hypothesized that the deficiency genotype would reduce athletic performance in sprint/power events.

Using the publicly available data from over 1000 individuals from 50 populations, Li et al. (2008) examined the between-population variation in the allele frequency of R577X. It ranged from near 0 in
African populations to over 0.8 in some American populations. The gene ACTN3 – the "speed gene", for fast-twitch muscle – makes muscles contract quickly. It's crucial for sprinters and sports requiring short, powerful movements. A variant (R577X) is needed for the slow, efficient muscle contractions required by endurance athletes, found in Type I muscle fiber types. A negative association between the ACTN3-deficient XX genotype and elite sprint athlete status has now been observed in six separate studies.

Based on these data, the scientific and sport community are interested in determining how much predictive power the R577X genotype provides in terms of identifying athletic potential? What role does the variation play in human health and fitness in non-athletes? In terms of statistics, individuals of European descent, less than a third of the population have two copies of the functional R allele (the RR genotype), while just over half the population has one copy of each of the two alleles (the RX genotype). Remarkably, the remaining 18% of the healthy European population—and in that regard more than a billion people worldwide—has two copies of the nonfunctional 577X variant (the XX genotype), resulting in complete deficiency of ACTN3 protein in their skeletal muscle (Mills et al., 2001). That fact suggests that ACTN3 can be compensated for by other factors, most likely including the closely related protein α-actinin-2.

In order to dissect the structural and biochemical changes underlying the effects of ACTN3 deficiency on muscle function, an ACTN3 knockout (KO) mouse was generated to serve as a model of human XX individuals (Chan et al. 2008). The KO mice were morphologically indistinguishable from their wild-type littermates, showed grossly normal muscle ultrastructure under light and electron microscopy, and did not demonstrate substantial loss of fast (Type 2B) fibers. Extensive studies were carried out on the effects of ACTN3 deficiency in a KO mouse model. If similar changes can be confirmed in human muscle, this transformation would provide a powerful mechanistic explanation for the negative association between ACTN3 deficiency and muscle strength and sprint performance, and also supports the notion that the loss of α ACTN3 may benefit endurance performance.

Several companies are already marketing ACTN3 gene tests directly to consumers. It would intuitively appear that testing R577X may be useful for coaches and sporting bodies, but how predictive will R577X genotype information really be? The answer to this question is still unclear, for a number of reasons. Firstly, many different genetic and environmental factors influence physical performance, with R577X genotype determining only a small proportion of overall variation. Cross section association studies estimated that R577X accounts for only 2.6% of the total variance in 40-m sprint speed in adolescent males (Moran et al., 2007). However, this percent can make a big difference for super-élite athletes. In addition to high motivation and world-class training, these rare individuals need to have a near-perfect set of genes to have a chance of winning an Olympic medal. These data emphasize that R577X is just one of a myriad of complex, interacting factors that influence muscle performance.

Myostatin is the growth factor that acts as a brake on the increase of muscle cell numbers. A person with a high levels of myostatin will have less well developed muscles and consequently less power (Hulmi et al 2009). A mutation in the gene for myostatin expression will remove this brake, and could lead to rapid muscle growth. A German toddler already has twice the muscle density of his peers – useful if he becomes a bodybuilder. However, this mutation only takes affect during embryogenesis but would be a direct indicator of muscle mass development after birth.

Insulin-like growth factor 1 (IGF-1) is the hormone most responsible for regulating cell growth and development predisposing height. An athlete with abundant IGF-1 and other growth hormones and regulators will be tall; useful for basketball players.

Super-mice have been genetically engineered to run faster and further. They over-express a gene for the enzyme phosphoenolpyruvate carboxykinases (PEPCK-C), which is modified so that it is only active in skeletal muscles. The mice produce less lactic acid and burn more fat. They can run at 20 m a minute on a treadmill for up to six hours.

Erythropoietin (EPO) is a hormone that regulates the numbers of red blood cells. Altering the EPO receptor enables blood cells to carry higher levels of oxygen, increasing the fitness potential. The effect is similar to that of blood doping.

Available Methods

Single-nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide in the genome sequence is altered. A high-throughput SNP array measures the hybridization of sample DNA to the templates of two different alleles for each SNP, and the relative hybridization strength
is used to call genotypes. Several commercial SNP chips, including Affymetrix GeneChips™ and Illumina BeadChips, can genotype over one million SNPs.

Protein separation methods require the extraction of proteins from control and experimental samples followed by processing for mass spectrometry analysis. Each sample usually contains thousands of proteins of different size and abundance therefore requiring extensive fractionation, enzyme digestion, and separation before mass spectrometry analysis.

A gene expression microarray is a glass or silicon chip attached with an arrayed series of thousands of microscopic spots of DNA oligonucleotides, each containing picomoles of a specific DNA sequence. After hybridization with fluorophore-labeled complementary DNA (cDNA) reversely transcribed from mRNA, the intensity of fluorescent light reflects the abundance of cDNA sequences and thus, indicates the abundance of mRNA sequences in the original mRNA sample. Gene expression microarrays simultaneously measure the expression levels of thousands of genes and produce a vast amount of data.

Gene-chip is a general term used to describe a new technology that can identify various allele polymorphisms or mutations. Analysing each polymorphism can be very time consuming, gene chips allow lots and lots of polymorphisms to be tested at one time very quickly. Affymetrix Inc. holds the registered trademark of GeneChip®, however this is not the only brand available.

Other molecular profiling platforms include proteomics and epigenetics analysis. The most common way to study protein modifications is via two-dimensional (2D)-gel electrophoresis. Mass spectrometry (MS) coupled with liquid chromatography (LC) and multiple reaction monitoring (MRM) has been successfully used to characterize and quantify proteins in complex mixtures. The two most common epigenetic events are DNA methylation and chromatin remodeling. Various experimental techniques have been developed for genome-wide mapping of epigenetic information.

Ethical Concerns
The use of gene-based technologies for TID poses an interesting ethical situation. Will such advancement only serve to further increase the chasm gap exists in elite-level sport between the nations with scientific funding, and those without? Or, how should the sports world react to such possibilities? Is it fair that those athletes who have access to a technology that could assist them in recovering more effectively and quicker, when others do not? Should the sports world be obliged to promote the utilization of such technology to optimize conditions of equality? Alternatively, does the utilization of such technology fall outside of what the sports world can assume as its regulatory responsibility?

Identifying the moment when genetic science becomes an ethical concern for the world of sport is difficult. Arguably, what distinguishes the ethics of genetic science in sport from other forms of scientific application is the way that it interfaces with a range of fundamental moral concern about the sanctity of life, human dignity, and what it means to be human (Miah, 2004).

Primary among the ethical concerns is the issue of athlete autonomy in the genetic screening process. The principle of individual autonomy suggests that if a person does not want to know about a particular disease susceptibility or condition, then he or she should not be forced into getting that information.

The idea of pre-birth selection is not new, but has previously relied on the old-fashioned technique of “matchmaking”. Patel and Greydanus (2002) raise the issue of “gene farming” where people may seek out “athletic” gamete donors in order to produce athletic genotypes.

Another consideration is if a child is aware that they may be lacking genetic potential for a certain sport, will they be less likely to participate? This was the question raise by Bouchard et al. (1997). The ALRC (1996) also raise the concern of genetic screening limiting life choices of those who are identified as “elite” at a young age, while discouraging others from even trying.

Australian Law Reform Commission (ALRC) examined the Disability Discrimination Act 1992 and concluded that genetic testing would not contravene this act providing the tests were “sufficiently reliable and relevant to the skills and abilities required” (p 5). ALRC report also cites the Australian Sports Commission Act 1989 that allows the AIS to reasonably discriminate between persons on the basis of physical and physiological attributes in order to implement a Commonwealth program. By its nature, TID needs to be discriminatory in order to select the best performers.

Considering recent stories in the popular press regarding genetic identification and international competition, the issue of ethics will continue to remain a central theme. As new technologies move forward, it seems that it will put continued pressure on athletes, coaches, counsellors, and physicians who will no doubt depend on the contribution of genetics to tell them something about athlete performance.

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Since the early 2000’s, debates about the ethics of genetics in sport have been dominated by the prospect of gene doping, the use of gene transfer technology for nontherapeutic or enhancing purposes. This technology promises a new era of performance enhancement in sport, which may call into question the possibility of detecting and catching users. Despite this possibility, WADA began to investigate the prospect in 2001 and responded in 2003 by prohibiting “gene” or “cell” doping within the 2004 World Anti-Doping Code (WADA, 2003) which read: “M3. Gene doping is defined as the non-therapeutic use of genes, genetic elements and/or cells that have the capacity to enhance athletic performance” (p. 14).

To this extent, questions remain about how genetic and molecular modifications or knowledge should be treated in the long term.

Conclusions
- TID is an important aspect of identifying potential elite performers, particularly in countries that don’t have the luxury of selecting from a large population base.
- The use of 1 gene as a guide for sport and event selection appears naïve given the complex interaction not only between genes, but between genetics and the environment.
- Genetic approaches appear most effective in individual sports rather than team sports.
- Perhaps the most likely use of genetic testing will be the identification of individuals with some inherent general capacities in psychological or motor skills that are transferrable across multiple domains
- More work needs to be done examining the influence and interaction of genes across a range of athletic parameters; and the effect of genetic screening on sport participation of children should be considered in greater depth.
- An enormous amount of research work is needed before we can predict the effects of an individual’s genomic and perhaps epigenomic characteristics on his or her ability to be trained and to reach elite athlete status in a given sport.
- Differences in human phenotypes result from the interaction of genetic variation with environmental stimuli. Further, such knowledge may be both translational and transferable—opening the way to the better understanding of disease processes (e.g., those causing progressive muscle wasting, bone weakness and fracture, or excessive cardiac growth).
- There will undoubtedly come a time when it will be scientifically possible to test for sporting aptitude but we have to decide whether we are happy with such tests and the conclusions we base on them.

References


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