Gene Doping: The Hype and the Harm

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KEYWORDS

• Gene therapy • Mutations • Detection • Polymorphisms
• Athletic performance

As genetic technologies and breakthroughs continue to progress at a rapid pace, so does the potential misuse of these advancements. Although ''conventional'' doping technologies are also evolving over time, one of the most intriguing and potentially destructive performance-enhancing concepts has arisen in the form of ''gene doping.'' Gene therapy has been established as a technique with the potential to correct nonworking genes that lead to disease, however, with only some success in human trials.[1] Adapting the principles of gene therapy to supply athletes with a competitive advantage is the (as of yet theoretical) goal of gene doping.

The World Anti-Doping Agency (WADA) categorizes gene doping as a ''prohibited method'' in its 2010 Prohibited List and defines it as ''1 - The transfer of cells or genetic elements (eg, DNA, RNA); 2 - The use of pharmacologic or biologic agents that alter gene expression… with the potential to enhance athletic performance.''[2] Although to date, confirmation of gene doping in competition has not occurred, WADA has acknowledged both the potential for misuse of gene therapy in this regard and the vigilance necessary to be ready to address this threat to fair play once gene doping moves from the realm of possibility to probability.[3] The allure of gene doping for an athlete looking to cheat is multifaceted but largely involves the inherent difficulty in detection.[4] However, although the unknowns associated with gene doping suggest detection difficulties, these same unknowns underscore the serious potential threat to the health and safety of the doping subjects.

METHODS OF GENE THERAPY OR DOPING

The general goal of gene therapy is to promote expression of a functional gene in an unhealthy individual to correct a disease caused by an underlying genetic mutation. The ideal gene therapy candidate is a monogenic condition caused by a nonfunctional or aberrant gene product, such as in Duchenne muscular dystrophy (DMD). In DMD, mutations in the dystrophin gene lead to absent, decreased, or dysfunctional

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dystrophin protein production. ''Classic'' gene therapy in this case would be the mechanism to deliver the dystrophin gene to an affected individual to produce functional dystrophin. This is the most common approach to gene therapy for monogenic conditions. ''Nonclassic'' gene therapy is the term used to describe this procedure when the goal of treatment is to control the expression of genes or the effects of gene expression, such as in cancer. Gene therapy is a promising tool in the treatment of genetic disease as it establishes treatment at the source of the underlying defect, and if continuous cell expression is achieved, it allows for a more constant administration of the gene product.[5,6]

To facilitate the introduction of a gene into the cells of the recipient, a delivery vehicle is required. These delivery systems can be classified into 3 main types: biologic, physical, and chemical.[7] The use of a biologic vector is the most common delivery mode for gene therapy. Viral vectors such as retroviruses, adenoviruses, and adeno-associated viruses (AAVs) are commonly used as they function to integrate into host cells and use this cell to replicate their own genetic material.[6,8] In gene therapy, these viruses are modified to reduce the potential for viral infection while carrying the ability to be delivered to specific cells for expression. Plasmid DNA (pDNA) is an alternative biologic vector but differs from viral vectors in that they are synthetic and may be grown in bacteria, then purified. Although more inefficient than viral vectors, pDNA has the advantage of avoiding a possible immune response.[5,6] Liposomes can assist in the penetration of cell membranes. RNA interference is another method of manipulating and controlling gene expression to enhance or manage gene therapy techniques that are under investigation.[6]

Gene transfer can be accomplished by direct physical injection or enhanced by physical methods, such as electroporation, ultrasound, laser, and magnetic particles.[5]

Gene expression can occur in vivo or ex vivo.[5,7] Both techniques have pros and cons, inside and outside of the laboratory (**Table 1**).

Of importance is the difference between somatic and germline gene therapies. Somatic gene therapy is limited to adult cells, and the effects are not a permanent change to an individual's DNA. Germline gene therapy, or transgenesis, is the process used in many animal studies and involves changing the genetic makeup of the animal permanently, including gametes, thereby making this genetic change present in all body cells and also heritable. Many of the animal studies in gene therapy that report the most significant results have induced germline mutations.[5]

The fundamental difference, physically, biochemically, and ethically, between gene therapy and gene doping is that the goal of gene doping is not to replace an absent or dysfunctional protein in an unhealthy individual but rather to artificially alter gene expression in an otherwise healthy individual. The evolution of gene therapy from a strictly medical tool to a performance-enhancement mechanism has significant ramifications both in the competitive sports world and in the general population.

CANDIDATES FOR GENE DOPING

What makes a gene a good candidate for doping? Obviously, the targets for gene doping would depend on the desired effect. Overexpression or underexpression of the gene product should enhance traits that are desirable for peak athletic performance. For endurance sports, such as long distance running or swimming, genes that bolster oxygen production or usage and delay fatigue would be the likely candidates. For sports in which strength or agility provide the competitive advantage, genes involved in muscle mass stimulation and injury recovery are the more likely targets.

Table 1
Ex vivo versus in vivo approaches to inducing gene expression

	Method	Advantages	Disadvantages
Ex vivo	Cells from patient treated in culture, then administered to the patient	• Allows for sorting and screening of gene product before expo sure to patient	• Low efficiency • Specific to an individual, which leads to higher costs, and the need for more specialized laboratories
In vivo	Gene is delivered via vector or direct physical route in to patient	• Allows for mass production of gene product • Lower costs	• Presorting or screening not possible • May cause immune response, which can cause efficacy, safety, and detection issues • Allows for possible germline integration

Research into gene therapy for disease treatment has led to a bounty of information that could theoretically be incorporated into gene doping programs.

Genes for Endurance

- *Erythropoietin (EPO)*: EPO is a hormone produced in response to decreased oxygen levels in the blood that signals the body to increase hemoglobin production.[9] EPO-stimulating agents have long been a part of performance-enhancing doping.[10] Overexpression of EPO by gene doping would increase endogenous hemoglobin production and thereby oxygen distribution to muscles.
- *Peroxisome proliferator-activated receptor delta (PPAR-δ)*: PPAR-δ and its family of hormones are involved in changing type I (fast twitch) skeletal muscle fibers to type II (slow twitch) muscle fibers.[9] Upregulation of this gene could produce an increase in the number of type II muscle fibers desired for endurance sports, even in the absence of endurance training. The WADA 2010 Prohibited List bans PPAR-δ agonists (eg, GW1516) and PPAR-δ–adenosine monophosphate–activated protein kinase axis agonists (eg, AICAR),[2] the only genes specifically mentioned under the gene doping section.
- *Phosphoenolpyruvate carboxykinase (PEPCK)*: the role of PEPCK in skeletal muscle is somewhat unclear, but overexpression in mice increases endurance and longevity and leads to decreased body fat.[7]
- *Vascular endothelial growth factor*: this growth factor is instrumental in the development of new blood vessels and also appears to be important in some injury-healing molecular pathways.[11,12]

Genes for Strength

- *Insulinlike growth factor 1 (IGF-1)*: IGF-1 is the primary target of growth hormone action. Increased gene expression leads to increased muscle mass and power.[8,13] In addition to promoting muscle hypertrophy, IGF-1 also hastens muscle repair.[8]
- *Myostatin*: unlike many other candidate genes for gene doping, myostatin would be targeted to promote decreased expression of this gene. Myostatin is a negative regulator of muscle growth, and by impeding its actions, increased muscle mass would be expected.[11]

Genes for Tissue Repair/Other

- *Bone morphogenetic protein (BMP)*: the BMP family of growth factors enhance bone repair and would theoretically shorten recovery time from injury. In the absence of an injury, these growth factors have the potential to increase bone, cartilage, or tendon strength in an effort to stave off potential career-ending injuries.[12,13]
- *Endorphins*: Endorphins are important components of pain management, fatigue delay, and endurance.[11] Genes that increase endorphins would increase pain threshold both acutely during competition by reducing lactic acid–related pain and chronically by dulling the effects of prior injury.[11,14] These effects make genes related to endorphin production, expression, and release reasonable targets for gene doping.

This is by no means a complete list of gene doping targets but an overview of prime candidates due to their cellular function. As more genes are identified and characterized with regard to athletic potential, the list of potential gene doping candidates is sure to expand as well.

GENE DOPING IN PRACTICE—ANIMAL MODELS

Animal models of gene doping have provided a wealth of information on the positive and negative effects of this procedure. Methods of successful gene transfer to adult animal cells have been demonstrated, and the successes of these transfers have been documented.[15] For example, gene doping with IGF-1 has proven successful in mouse models, whereby a discernible increase in muscle mass and strength was noted even months after the treatment concluded. These same studies showed that combining gene doping with athletic training provided a significant advantage over nontreated controls.[16] PPAR-δ transgenic mice showed an increase in running time, longer endurance, and lower likelihood of obesity.[7] EPO gene doping in macaques has demonstrated systemic effects, including increased aerobic capacity, improved performance, and elevated hematocrit levels.[17] Follistatin, an inhibitor of myostatin, has been used in gene therapy trials with AAV vectors to create increased muscle bulk in a variety of animal models.[18]

However, in addition to shedding light on the potential enhancement effects of gene doping in humans, animal studies have also uncovered concerns that may directly affect human subjects. It is perhaps unsurprising that artificially overexpressing genes to promote athletic prowess may lead to unwanted and negative side effects. Some studies reported an increase in hyperactivity, aggressiveness, and other behavioral sequelae in treated mice. Overexpression of EPO in macaques has been reported to increase blood viscosity, with effects on cardiac functioning.[7] Clearly, although animal models may demonstrate the promise of gene doping, the perils of this procedure cannot be ignored because its use is contemplated in humans.

GENE DOPING IN THEORY—HUMAN MODELS

Although animal studies have been successful in demonstrating gene doping effects, the transfer of this technology to humans is met with considerable logistical and practical limitations. In mouse models, high vector doses were required to induce significant effects. It is not clear how high a vector dose would be required for human gene doping or if humans have a similar capacity to tolerate these vector doses safely and effectively.[5] Are current laboratory techniques and resources capable of handling these challenges? Gene doping targets would also need to have a sustained and

regulated response to produce results similar to those in animal studies.[8] In addition to these size and distribution considerations, a potentially more serious limitation is that of control of gene expression. Successful gene doping would require tissue-specific responses. How to reliably turn gene expression on or off is critical for safety, performance, and detection purposes. Uncontrolled gene expression has the possibility of actually reducing performance,[13] making the entire gene doping procedure counterproductive.

Performance Mutations and Polymorphisms

Perhaps the first human models that demonstrate the potential for gene doping could be those with naturally occurring mutations that lead to altered gene expression. For example, Finnish cross-country skier Eero Mantyranta won 2 Olympic gold medals in 1964, far surpassing his competition. Ultimately, he was found to have a naturally occurring mutation in his EPO receptor gene that vastly increased his endurance by increasing hemoglobin levels.[7] Another case of naturally occurring performance-enhancing mutation has been documented in an extremely muscular child who was found to have loss-of-function mutations in both copies of the myostatin gene. The inactivation of this gene led to excessive muscle bulk and strength with neonatal onset.[19]

In addition to the significant effects of a single-gene mutation on athletic performance as described earlier, researchers have also begun to identify dozens of genes involved in athletic ability. Subtle variations in these genes may naturally predispose an individual to certain types of physical activities. It is fascinating to consider not only our individual genetic predispositions to "performance- and health-related fitness phenotypes"[20] but also the impact that the discovery of these genes could have on the world of gene doping. The number of candidate genes reported to be associated with endurance, muscular strength, power, body composition, training response, and other athletic traits is continuously increasing.[20,21] For example, a specific genotype in the angiotensin I–converting enzyme (ACE) gene results in reduced serum and tissue ACE activity. This genotype has been found naturally in a higher than expected rate in elite endurance athletes.[21] Nutrigenetics, or the interaction between food or supplements and genetic predisposition, is another area of interest where genetics and sport interact with positive and negative potentials.[11]

Gene doping is not the only possible method of abusing our newfound knowledge in the genetics of athletic performance. Studies have also shown that genetic variability in the ability to process testosterone can exploit weaknesses in current doping detection strategies. A specific polymorphism in a gene-involved testosterone metabolism (*UGT2B17*) can result in decreased levels of testosterone glucoronide being expressed in urine. This creates a problem in the detection of testosterone abuse in individuals carrying this natural gene change.[22] Knowledge of one's specific genetic ability or inability to process metabolites used in doping detection could be exploited to the advantage of the dishonest athlete. Genetic testing for such polymorphisms could become necessary as doping detection programs attempt to stay up to date with genetic knowledge and technology. These complicated practical and ethical issues raised by the identification of genetic polymorphisms have been considered by WADA, and the importance of staying current on these developments was addressed at their 2008 symposium.[3]

As more genes are identified and further characterized, the possibility of genetic testing for athletic aptitude is gaining attention in academic circles and the popular media.[23] It is therefore not unreasonable to suspect that the combination of genetic predisposition testing to determine suitability for a specific type of sport could be

combined with gene doping in attempts to create a "super athlete." Although there is no evidence of such activities at the current time, the athletic world will not remain untouched in the era of personalized genomics.

RISKS OF GENE DOPING

The risks associated with gene therapy in a regulated, controlled setting are still being defined. Results of gene therapy trials performed in the 1990s indicated both a substantial variability in response to vectors and a nonlinear relationship between vector dose and toxicity. The death of an 18-year-old volunteer in a pilot study of gene therapy was attributed to systemic inflammatory response syndrome caused by an immune response to the adenoviral vector used.[24] Therefore, the risks associated with taking a new procedure and illegally abusing it in otherwise healthy individuals are real and concerning. The most significant risks are associated with both the unregulated delivery of the gene therapy by dopers and the effects of this doping on a cellular and functional level in the athlete.

The illicit production and administration of gene doping products would compound these risks. The safety, quality, and contents of a gene doping product would be unregulated, and the secretive nature of doping in general may hinder appropriate medical follow-up if needed.[11,21]

The risks secondary to altered gene expression include (1) an immune response to a viral vector, (2) an autoimmune response to a recombinant protein, (3) insertional mutagenesis, and (4) lack of expression control and the sequelae related to an artificial overproduction of a protein in a healthy subject.[5,6,8,13,17] The immune response to a viral vector can be mild, such as a fever or inflammation, but could also be overwhelming and fatal.[5] If a protein produced differs from that which is endogenously produced, autoimmune responses are possible. The prospect of insertional mutagenesis is concerning in that the vector could insert itself into the host genome and disrupt oncogenes, leading to tumor development. In some cases, the risk of germline integration, or permanent, heritable genetic changes, being introduced adds another serious ethical consideration to gene doping.[6,17] EPO overexpression leads to increased blood viscosity, which can increase the risk for heart failure or stroke.[5] Overexpression of a growth factor, such as IGF-1, can cause cardiac hypertrophy and stimulate growth of cancerous cells.[8] Increasing muscle mass by manipulating myostatin, IGF-1, or other factors is also likely to put extra stress on supporting bones and tendons, which could actually increase the risk of injury.[12]

DETECTION STRATEGIES

The only way to address the possibility of gene doping detection is to stay current with scientific techniques and potential avenues for abuse of gene therapy. WADA included gene doping in their list of banned methods in 2003, continues to monitor developments in this area closely, and sponsors research into detection strategies.[3] To be successful, doping detection needs to be accessible, fast, and reliable: 3 significant challenges when dealing with gene doping. For example, if a gene doping product is produced by introducing genes to make more proteins endogenously, how can it be distinguished from the naturally produced protein? Can evidence of gene doping be reliably assessed using body fluids? Studies have shown that gene therapy with IGF-1, while increasing the detectable levels in muscle cells, did not show an increase in circulating IGF-1 levels in the blood.[5,8] A muscle biopsy, although more sensitive, is not practical. Detection strategies can be categorized into direct (evidence of doping agent) and indirect (evidence of consequences of gene doping).

Direct Approach

Direct detection of gene doping would involve identification of the vector used or a recombinant protein that differs from naturally occurring protein. Vectors have been identified in blood after gene therapy, but the window of opportunity for detection seems to be short, which poses obvious limitations.[13] There is some evidence that some proteins produced by gene therapy undergo slightly different posttranslational modification, which opens a possible detection. Some genes may be regulated by promoters that need to be activated. Detection of activating substances such as rapamycin, tetracycline, and antiprogestins would indicate gene doping but may also be present for therapeutic reasons.[5] Although direct evidence of gene doping may be preferred, especially if legally challenged, the technical limitations of these processes may not make it the most likely solution for gene doping detection.

Indirect Approach

Indirect detection of gene doping would involve the identification of the consequences of this procedure on the athlete. Various fields of study are being investigated as a potential ''biologic profile'' to distinguish normal standards from those indicating gene doping. For example, transcriptomics, or the profiling of gene expression, measures changes in the concentration of messenger RNA for thousands of genes. Proteomics, or protein profiling, evaluates the set of proteins expressed from the genome and provides qualitative and quantitative analysis of their variants. Metabolomics, or the profiling of nonprotein low-molecular-weight metabolites, can also provide a possible measure of gene doping activities.[4] Although these are promising approaches, the development of normal standards, individual ''passports,'' and variability parameters is expected to be costly, time consuming, and open to legal interpretation if a gene doping charge is made. As illustrated in the testosterone metabolism studies, natural genetic variation in the population can lead to extremely variable enzyme activity under normal circumstances and may provide enough reasonable doubt to discount a suspected case of gene doping.[25]

SUMMARY

The advancement of genetic technologies that have lead to the exciting treatment possibilities of gene therapy has also opened the door for their abuse as a performance-enhancing agent. Although gene doping may be a desirable cheating method because of the inherent detection challenges, its effects are still largely unknown and potentially lethal.

The physical, ethical, and societal pitfalls of performance-enhancing doping are numerous. Gene doping adds another level of concern not only in the sports world but also for society in general.[26,27] If gene therapy has potential for abuse in sports, it also has the potential for abuse for nonelite athletes looking for a competitive edge or even people who would like to bulk up their muscle mass or lose weight by altering their genes.

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