

## Editorial Review: "Gene doping in sports: A medical perspective."

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### Abstract:

In the recent years, there are remarkable advancements in the scientific knowledge and technology in the field of genetics. Especially, gene therapy has shown the entirely new modality to treat many earlier non treatable conditions by manipulating with the genetic sequences. However, these advancements also highlighted and raised the possibility of its potential misuse in sports. These gene manipulations can be used in healthy person to enhance the performance. Several animal trials have also successfully demonstrated the improved muscle mass, strength and endurance of laboratory animals through gene manipulation techniques. In response to this raised concern on gene doping, without a single incidence far yet, World Anti Doping Agency (WADA) in 2003, declared gene doping illegal and prohibited in sports. WADA defined gene doping 'as non therapeutic use of genes, genetic elements and /or cells that have capacity to enhance athletic performance.' The present review is an attempt to discuss this issue of gene doping from the medical perspective. The present review will discuss gene doping concept, its scientific basis, possible target genes, detection methods, risks, preventive measures and ethical issues.

Keywords: Gene doping, Myostatin gene, EPO gene, IGF-1, HIF-1.

**Introduction:** Sports is an activity involving physical exertion and skill in which an individual or team competes against each another or others for entertainment. Fair-play or playing true is a spirit of any sport. But now-a-days sport has become a major business and sportsmen perform not only for an eternal fame and honour but also for money. In many sports, high performing participants are rewarded with very lofty pays and lucrative

advertising contracts. Therefore, not surprisingly, to win the race to be best, many professional sportsmen are being involved in unethical and unfair means. For decades, professional sports have been smudged by doing which is the unethical and non medical use of performance enhancing drugs by the sportsman. World Anti Doping Organization (WADA), in 2004 Olympic Games in Athens, implemented the World Anti Doping code, to harmonize the rules and regulations governing anti-doping across all sports and all countries.

In the recent years, there is remarkable advancement in scientific knowledge and technology especially in the field of genetics. Last decade has witnessed major

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milestones like the publication of human genome project and successful gene manipulation methods to treat various diseases in humans. Several animal trials have also demonstrated the improved muscle mass, strength and endurance of laboratory animals through gene manipulation techniques. [1, 2] Thus gene manipulations can be used in healthy person to enhance the performance. In these days there is the raised concern over issue of use gene doping in sports.

The present review is an attempt to discuss this issue of gene doping from the medical perspective. The present review will discuss gene doping concept, its scientific basis, possible target genes, detection methods, risks, preventive measures and ethical issues.

### **Gene doping: Concept and Scientific basis:**

Gene therapy involves replacement of defective / abnormal genes by introducing the desired gene into the appropriate cells of the patient. Many highly efficient gene delivery systems like retroviral vectors and plasmids have been developed recently. Diseases in which gene therapy has already been successfully include Parkinson's disease and many other immunological disorders.

Apart from the treatment of these diseases gene therapy may be used in healthy persons with potential application to enhancements sport

performance. In 1997, Svensson EC et al delivered the erythropoietin gene in mice and monkeys with improvement in haematocrit up 81%. [3] Transgenic mice created by McPherron in 1997 and Philadelphia based researchers Barton-Davis ER et al in 1998 demonstrated enormous increase in muscle mass and strength. These mice were baptized as 'Schwarzenegger mice'. [1, 2]

Following the raised possibility of its use in human athletes, the medical committee of the International Olympic Committee and World Anti Doping Agency (WADA) organised different meetings in 2001 and 2002 to discuss issue of gene doping. Finally in 2003, WADA defined gene doping as 'the non therapeutic use of cells, genes, genetic elements or the modulation of gene expression, having the capacity to enhance athletic performance.' and notably without a single incidence so far, declared it as illegal and prohibited in sports.

### **Potential target genes for gene doping:**

#### **1. Growth hormone (GH):**

Growth hormone has multiple growth promoting effects on the connective tissue, muscle and tendon. Also it has stimulatory effect on carbohydrate and fat metabolism. Recombinant GH, which is produced using genetic technology, is already being used as a doping agent and policies have been made to detect the same.

The gene encoding the GH can be directly introduced in humans to increase the intrinsic production of GH and detection of such gene cheating will be very difficult. This technique was originally devised to treat patient with GH deficiency but it has the potential to be used by the athletes to improve their performance.

#### **4. Insulin like growth factor – 1 (IGF-1):**

It is also known as muscle growth factor which is a 70 amino acid polypeptide, mainly synthesized by liver and in small amounts by the skeletal muscles. It stimulates the growth and differentiation of skeletal muscles and bones. [4]

In several animal trials, gene therapy IGF-1 is has been successfully used to strengthen the muscle in degenerative muscle conditions such as muscular dystrophies. Musaro et al in 2001 demonstrated marked muscle hypertrophy and increased injury healing response in mice induced with IGF-1 gene. [4] Similarly, Barton-Davis et al [2] and Lee et al [5] showed IGF-1 gene therapy counteracted the age related muscle atrophy in addition to increase bulk both in response to training as well as in absence of any special exercise.

In an athlete, IGF-1 genes may be inserted in skeletal muscle to get muscle hypertrophy with increased strength and endurance. Plasmid or viral vectors can be used to deliver this gene

locally. [6] Such therapy is likely to be more specific and safe as it acts locally at the targeted muscle. Examples include strengthening shoulder muscles of tennis players, biceps of boxers and calves of sprinters. Human trials of this gene therapy will start soon.

#### **2. Myostatin :**

It was first time described by McPherron et al [1] as a strong negative regulator of muscle growth and differentiation. It is synthesized by the skeletal muscle itself and acts locally. Mice in which myostatin gene has been inactivated showed enormous increase in muscle mass with less fat and connective tissue. Mosher et al performed artificial mutation in myostatin gene in racing Whippet dogs and resulted in ‘double muscled’ dogs with significantly less fat mass. These dogs showed markedly improved racing performance. [7] As reported recently, a child carrying genetic mutation in myostatin gene also had similar enormous muscular hypertrophy. [8]

Same as the IGF therapy, myostatin gene blocking also can be carried out locally. Thus it can be used by many athletes to improve strength of particular muscle groups. Also it can be used by the bodybuilders as it leads to exaggerated musculature with decreased fat deposition, giving person a lean, sculpted appearance.

#### **3. Erythropoietin (EPO):**

A good oxygen supply to skeletal and cardiac muscle is extremely important for the athletic performance. EPO is a hormone secreted by kidneys and in small quantities by liver. It acts on bone marrow to increase erythropoiesis which leads to increase RBC mass. The studies carried

out in mice, rabbits and monkeys analyzed the efficiency of intramuscular injection of EPO gene. [9, 10] Athletes can use this drug to increase their endurance hence WADA have already prohibited its use in sports.

#### **5. Vascular endothelial growth factor (VEGF):**

Another possible gene doping is inserting VEGF gene in athletes to enhance the growth of new blood vessels in skeletal and cardiac muscle from the pre-existing blood vessels (angiogenesis). This will lead to a marked increase in microcirculation and delivery of oxygen and nutrients at the tissue level. Because of this there will be marked improvement in the endurance and injury healing. Clinical studies have demonstrated its efficiency in patients of angina [11] and peripheral arterial diseases. [12] As clinical trials are already successful in humans using viral vectors, presently VEGF gene therapy has greatest potential to be used as a gene doping.

#### **6. Hypoxia inducible factor (HIF-1) :**

The recent discovery of hypoxia inducible factor has increased the understanding of tissue response to lower oxygen. [13] HIF is the novel family of transcription factors which modulates variety of genes in response to hypoxia. It has shown to increase transcription of erythropoietin and vascular endothelial growth factor. Both these mechanisms are pivotal in increasing athletic endurance and tissue injury healing. HIF gene has also

demonstrated increase in some glycolytic enzymes; which produces additional energy in conditions of relative oxygen deficiency. Researchers have also developed novel vectors to target HIF gene. [14]

#### **7. Endorphins:**

Pain is the protective phenomenon. However, in athletes, pain due to injury or muscular exhaustion (accumulation of lactic acid) significantly reduces the ability to perform. Use of analgesics potentially helps athletes to improve their performance. This advantage of pain relief has brought several candidate genes into limelight for gene doping. The gene coding for endorphins and enkephalins could be administered, targeting central nervous system and locally, for increasing the threshold for pain. Preclinical animal studies have successfully tested these effects. [15] This promising pain killer gene therapy is still in the primitive stages of development.

#### **8. Leptin :**

It is adipose derived hormone which regulates energy intake, expenditure and hunger. Murphy et al in 1977 [16], injected virus carrying gene for leptin in mice and demonstrated an increase in lean body mass. The results of this experiment are promising and needs further exploration in humans.

#### **9. Peroxisome proliferators activated receptor $\delta$ (PPAR- $\delta$ ) :**

Activation of PPAR- $\delta$  in skeletal muscle has proven

to increase the endurance capacity. Wang et al in 2004 showed that transgenic mice with expression of PPAR- $\delta$  gene had doubled the endurance [17]. In elite cyclists, skeletal muscle mRNA expression of PPAR- $\delta$  was associated with skeletal muscle fibre types having more endurance. [18] Thus gene transfer PPAR- $\delta$  gene might improve endurance of athletes.

### **Limitations and possible risks associated with gene doping:**

There are some limitations to its therapeutic use and application.

1. Gene transfer technologies are not efficient and safe enough to be used for most of its applications.
2. Till date, the most impressive results were demonstrated in lower animals like mice. Mice are small creatures and many successful trials used very high doses of vectors in them. In human, whether the same results will be achieved at proportionately higher doses of vectors is still unanswered question.
3. Gene expression of transferred gene is not very well understood yet. It's switching on and off is not under our control.
4. Therapeutic gene transfer technologies carry unforeseen and unresolved risks.

The possible risks with gene doping fall into 3 main areas: general, special and environmental risks.

### **General risks:**

So far, over 3000 trials have been conducted to treat patients with gene therapy. The products and procedures used for delivery of genes carry some risk. Few of these general risks are

- Some clinical trials reported morbidity associated with viral vectors and one death due to overdosing of vectors in 1999. [19]
  - Two patients of immunodeficiency disorders, who were cured with gene therapy, in later dates developed leukaemia. [20]
  - Recently, an autoimmune response was reported with adeno-associated virus vector use for EPO gene delivery.[20]
  - Other reported side effects of gene therapy are flu-like symptoms.
  - The gene transfer vector may get contaminated during its process of manufacturing. Then there are high chances of generating a totally new virus. This may impose a great risk to recipient of gene therapy as well as to the community.
- **Special health risks:**
- Uncontrolled expression of the genes used in gene doping carry potential health risks similar to the other forms of doping. Some of the examples are

- Both GH and IGF-1 are reported to be potent mutagens and anti-apoptotic agents. It carries increased risk of malignancies.
- Localized use of myostatin blocking therapy might lead to development of disproportionately large and strong muscle. And due to its excessive and inappropriate pull, there are chances of tendon tears and bone fractures.
- Uncontrolled over expression of EPO genes carries risk of thrombo-embolism, stroke and cardiovascular accidents due to increased haematocrit.
- Over expression of VEGF, HIF and other angiogenic factors can increase the vascularisation of developing solid tumours and carries potential risk of promoting cancerous growth.

#### **Environmental risks:**

After therapeutic gene therapy, there are high chances of excretion and spread of gene transfer vectors through body fluids and excreta of the gene therapy recipients. These vectors have potential to infect and get transmitted to the contacts. The current gene therapy experiments are closely monitored. But the possibility of such transmissions cannot be excluded, when athletes will be using gene doping in uncontrolled environment. This may pose a serious environmental risk.

#### **Possible methods for detecting gene doping and its challenges**

Artificially manipulated genes are likely to be identical to the naturally occurring genes and its products. Hence detection of gene doping will be difficult. Although, it could be accomplished by several methods as follows:

- The easiest approach is to apply molecular tests to detect insertion vectors in the plasma and other body fluids of a person. But these vectors (Plasmids and virus vectors) have very short half life. Therefore, we need to carry out these tests with relatively shorter intervals and with regular testing regimes.
- ‘Genetic bar code method’ or labelling of gene transfer products can be done. This is already being practiced with genetically modified agricultural products. To develop this approach there is a need for the complete cooperation of scientists, ethicists, athletes, sports authorities, medical practitioners, professional societies and none the less, public.
- The engineered genes do not contain introns. This fact can be used in detecting gene doping by using molecular tests. But this method will involve tissue sampling like muscle biopsies. Due to the invasive nature of this test, it is very unlikely to be accepted by athletes and

and sporting authorities.

- There are some structural differences in endogenous and recombinant EPO. EPO gene doping can be detected using this method. [21]
- The gene therapies are ultimately targeted to produce some or the other human protein. Sampling, analyzing and maintaining the set of protein level's data from individual athletes over times, is another possible solution. This will require profiling individual athlete frequently and regularly for years, which would impose additional regulatory and cost burden.

Thus due to these challenges and limitations with current technologies, there is no way to detect gene doping with high sensitivity and specificity.

### **Suggestions and recommendations**

- Today, there is a need to promote the research and developments of detection method, at a global scale.
- Athletes should be properly informed and educated about the possible risks and consequences of gene doping.
- The clinical use of gene therapy should be strictly regulated to prevent doping.

**Other side of coin :** If in the future, gene therapy becomes adequately safe to be used therapeutically, it will be accepted as a standard medical treatment. Many diseases and disabilities like muscular dystrophies will be cured completely. This will make the disabled people able to compete at higher levels. These techniques also have a potential of healing the injuries better and accelerated way. Then the question will be raised, how can we deny an athlete for using these advances technologies? Just because he is an athlete, will it be ethical to prohibit him from a standard medical treatment?

So should we go ahead and make gene doping legalised? Then it will be like designing people for the sports. Professional sports will resemble like formula one race with team of biologists at the background. We might lose the joy and the love for the games.

**Conclusion:** Presently, gene therapy methods are successfully being evaluated in many clinical trials to treat various diseases. Most likely within next 5-10 years it will be the standard therapeutic treatment method. Then there is high possibility that these methods will enter in the arena of sports for unethical performance enhancement. Already, we have adequate knowledge about the potential gene for performance enhancement. Sooner or later, the athletic world will face the serious issue of gene doping.

The present day detection methods have failed to detect doping, which affects the scenario even more badly. This future uncontrolled use of gene doping will impose potentially serious risks both for the user and the environment. WADA has already declared the gene doping prohibited in the sports. They are doing all the right things to move ahead in the game of gene doping. But, where existing regulations on use of gene therapy are sufficient to tackle the future uncontrolled use of it, is unanswered question. Development of detection methods at the global scale, educating the athletes regarding the risks of gene doping and re-evaluating the existing regulations for gene therapy are the few preventive measures which seems to be promising at this moment. These measures might just stop the science from murdering the sports.

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