GENE THERAPY AND ITS IMPLICATIONS IN SPORTS

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SUMMARY

Thanks to the very successful Human Genome Project and the identification of genes involved in genetic disease, we now have the ability to treat many conditions. However, the identification of the genes which code certain phenotype characteristics has opened the way for abuse in the fields of sport and physical exercise. The principles of gene therapy and the ways in which genes are transferred have completely been copied from gene therapy and are now being used to increase the physical abilities of athletes. The genes most frequently used by athletes include: the the ACE gene, the ACTN3 gene, myostatin, the erythropoietin gene, PPAR-delta and the like. The misuse of these genes with the aim of increasing physical abilities has already become part of sport and is extremely difficult to identify, since genes and gene sequences entering the human body are proteins that are already structural and functional parts of the organism. On the other hand, viral vectors as the instruments for gene transfer attack and destroy the human immune system, and the reaction of the human body can be negative, with a danger of insertional mutagenesis and the appearance of oncogenes. Gene therapy might actually be much more useful in treating sports injuries, but even these procedures are still far from clinical practice. There is a fine line between gene therapy and gene doping in athletes. A number of growth factors will enhance repair, but it happen that expression of these factors increase the strength of bones and tendons, so that giving an advantage to competitors. First of all, it is necessary to acquaint athletes as much as possible with the negative consequences of using gene therapy. However victory and glory may be strong achievements, the health of these young people, and respect for fundamental and ethical principles, humanity, and fair play game have a more lasting value and represent the heavier weight on the scales.

Key words: gene transfer, candidate gene, performance enhancement.

INTRODUCTION

During the last decades of the 20th century, modern medical science has undergone an extraordinary expansion in terms of new discoveries. The greatest achievement has been the discovery of the genetic factors responsible for certain conditions. The international program for sequencing the entire human genome was started in 1990, and a complete DNA sequence was released in 2004. Prior to that it was believed that there were around 100.000 genomes which code proteins and determine what human life will look like, but with this project a much smaller number of genes has ben discovered, somewhere between 25.000 and 30.000. The direct benefit to be drawn from these data is the ability to make more precise diagnoses in the case of genetic disorders and the development of new strategies for treating monogenetic and polygenetic disorders.

Gene therapy is a medical procedure which uses nucleic acids for the purpose of replacing or completing damaged genes. Gene therapy was first tried out on humans in 1990, and since then this method has been used on over one thousand people over the world. Synthetic or recombined DNA is used, genetically modified stem sells, bare nucleic acids, the antisens technique (for example, "inhibiting" genes, the correction or modification of genes), genetic vaccines, RNA interference, and xenotransplantation of animal cells (Baoutina, Alexander, Rasko, & Emslie, 2007). Nevertheless, even though the progress made in gene therapy is evident in terms of conventional therapy, its practical application is still limited, since

it is encumbered by many difficulties. The fact that the gene therapy carries a great risk with it is confirmed by the data that three out of eleven children, for example, who received gene therapy for severe combined immunodeficiency related to the X chromose, became ill with leukemia, which resulted in the death of one of the children (Cavazzana-Calvo, Lagresie, Hacein-Bey-Abina, & Ficher, 2005).

One of the basic prerequisites for gene therapy is the cloning of a given gene. This refers not only to the structural gene, but also to the segments of DNA which participate in the regulation and expression of that gene. The next step is to determine the specific target cells, tissue or organ, as well as to determine the way the gene will be introduced into the human body. Gene therapy is usually preceded by research involving animal models, which has led to the creation of the cystic fibrosis model, the Duchenne muscular dystrophy, Huntington's disease, and Friedreich's ataxia.

Gene transfer into the target tissue is carried out in two ways: *ex vivo* (outside of the body) and *in vivo* (within the body itself). In the case of *ex vivo* transfer, the cells or the tissue which have been taken from the patient are treated and then returned into the body of the patient. *In vivo* gene transfer is the most direct means of gene transfer and in theory can be used to treat many hereditary disorders.

There are two basic types of gene transfer: *viral* and *non-viral*.

Viral vectors – A great number of different viruses can be used to transfer genetic material into cells.

- *Oncoretroviruses* belong to the group of RNA viruses which can be integrated into the DNA of the host, by making a copy of their RNA using the enzyme for reverse transcriptase. The provirus that is thus created represents a matrix for the creation of iRNA for various viral proteins and the new genome RNA of the virus. If the virus is in a stable manner introduced into the stem cells which will further continue to divide, all of the cells that will develop from them will inherit a copy of the virus genome. One of the problems that occur during the use of a retrovirus as the vector system is that they can accept and transfer into the target cells relatively small segments of DNA, which limits their use, in addition to the fact that they can be integrated into cells immediately following an infection, while the number of cells which is continuously being divided is small.
- *Lentiviruses* are complex viruses which infect macrophages and leukocytes and can also be integrated into cells which are not undergoing a process of division.
- *Adenoviruses* are suitable for gene therapy because they can infect a great number of different cells and can carry larger segments of DNA. The unwanted sideffects of their use are the stimulation of the immune response of the host, in addition to the possible malignant characteristics of the virus itself.
- *Herpesviruses* are mainly used for targeted gene therapy of the CNS and for treating neurological disorders such as Parkinson's disease. Their flaw is their potentially toxic effect on the cells of the nervous systema and the induction of an immune response, in addition to poor integration and instability.

Non-viral vectors – The advantage of these vectors is that they do not cause an immune response, they are safer and simpler to use, but their effectiveness is limited.

- *The DNA itself* is often directly inserted into the cells, but its use is limited only to the expression of hormones or proteins which in small doses can have a significant clinical effect (for example, erythropoietin).
- *Electroporation* consists of application of a high voltage current to target cells. This causes the pores on the cell membrane to open, so that the exogenous DNA can enter the cell.
- *The "gene gun"* is a device for injecting cells with genetic information. Heavy metal (gold, silver, wolfram) particles are used, coated with plasmid DNA. These particles are delivered to the target tissue with the help of a helium propellant, and the DNA reaches the nucleus of the target cell and is integrated into the genome DNA.
- *The transfer of DNA by means of liposomes* – liposomes are vesicles with a liquid content surrounded by a two-layer lipid shield, which can facilitate the delivery of exogenic DNA into the target cell. Their flaw is their less efficiency in gene transfer, and their advantage is that they can enter the cell larger segments of DNA from viral vectors.
- *Receptor-mediated endocytosis* represents a type of gene transfer using liposomes, in which the target is receptor on the cell surface. The success of this way of transferring the gene can be increased if the gene products include adenoviruses or influenza viruses.

Somatic stem cell therapy – Stem cells are unspecialized cells which are defined by their capacity

to reinvent itself and their ability to differentiate into specialized cells of many cell lines. Bone marrow transplantation is a form of somatic stem cell therapy, but is accompanied by a risk of infection due to immunosuppression and graft versus host disease.

Stem cell transplantation (for example, pluripotent hematopoietic stem cells) in utero offers a perspective for a new way of treating many genetically conditioned disorders. The immaturity of the fetal immune system allows tolerance of foreign cells, and do not require similarity between the donor and fetal cells.

Embrionic stem cell therapy – These cells originate from embryonic cells during the blastocyst phase, and are pluripotent, which means that they can develop each of the three germ layers, or all the cell types that exist in an adult human body. These cells can also be used in gene transfer technology as a "vehicle" for genes which partici-pate in the correction of the phenotype.

Gene therapy, as a clinical reality, is still in the stages of development. Even though the clinical circumstances of the work are higly controlled and regulated, the patients are exposed to great risk and therapy failure. Success also depends on the type of vector used, the vector administration procedures, the immune response of the body, the gene expression, as well as the potential oncogenic effect, depending on the mutation of normal genes as well as the result of the integration with the host genome.

THE IMPLICATIONS OF GENE THERAPY IN THE FIELD OF SPORT

Thanks to the success of the Human Genome Project, we now are facing the possibility of identifying genes involved in genetic disorders such as diabetes, Alzheimers disease, Parkinson's disease, muscle dystrophy and the like (Turnpenny & Ellard, 2007). Nevertheless, instead of solving certain problems and treating certain diseases, the discovery of the genes responsible for certain physical characteristics has brought whole series of possibilities for manipulation in areas such as sport and physical exercise.

A great many factors determine sport success: genetics, training, diet, motivation, the advantage of proper sports equipment, and the like. Genetics has a great influence on the components of sports performance such as strength, endurance, muscle fiber type distributions, anaerobic threshold, lung capacity, neuromuscular coordination, temperament and the like. It is a fact that physical ability, determined through innate physical characteristics, irrespective of training and diet, has been the cause of much research interest, so that the genes which determine certain physical abilities have become an object of study. Despite the possible role of genetics in the phenotype of elite athletes, the number of genes which are thought to influence the level of physical abilities is not great, since genetic preconditioning is polygenic, which means that more genes with a smaller effect can determine certain physical characteristics (Brutsaert & Parra, 2009).

Genes, which are assumed to be the cause of a characteristic phenotype based on their function, are *gene candidates*. The list of gene candidates for the improvement of physical abilities is not final, as is constantly being updated with new genes, while an entire group of other genes which code metabolic enzymes is being analyzed.

The principles of gene therapy and the means of gene transfer have been completely borrowed from gene therapy and are being used to increase the physical abilities of athletes (Vitošević, 2011).

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

The genes most frequently used by athletes are: the ACE gene, the ACTN3 gene, myostatin, the erytropoietin gene, PPAR-delta, and the like.

The ACE (Angiotensin-Converting Enzyme) gene – has a key role in the regulation of the renin-angiotensin-aldosterone system. The ACE catalyzes the conversion of angiotensin I to angiotensin II and has an important role on electrolyte balance and systemic blood pressure (Rieder, Taylor, Clark, & Nickerson, 1999) and has also been proven to have a role in the degradation of bradykinin which can inhibit growth. Skeletal muscles have their own renin-angiotensin system, which can be important for tissue growth and muscle hypertrophy. In this case we can assume that genetic variation in the system leads to differences in muscle mass phenotypes, making ACE a candidate for sprint or power performance. Most of the data indicates polymorphism, which is characterized by the presence (insertion, I allele) or absence (deletion, D allele) of 287-bp sequence in intron 16, resulting in three genotypes: II and DD homozygote s, and ID heterozygous.

Data that can be found in the literature regarding the roles of certain alleles in the ACE enzyme geno-

type are controversial. By studying the connection between the presence of different alleles and elite sport, various data have been obtained. For example, the greater presence of I alleles has been noted among athletes involved in higher-endurance sports, long-distance running, mountain climbing, diving and the like, while the presence of D alleles was greater in sports involving strength, sprinting, short-distance swimming and the like (Costa, Silva, Breitenfeld, Marques, Marinho, Garrido et al, 2008; Nazarov, Woods, Montgomery, Shneider, Kazakov, Tomilin et al, 2001; Tsianos, Sanders, Dharait, Humphris, Grant, & Montgomery, 2004; Woods, 2009). Several studies have failed to identify any association between the ACE I/D polymorphism and elite human performance. Taylor, Mammote, Fallon, and Bockxmeer (1999) examined 120 Australian national athletes from sports deemed to demand a high level of aerobic fitness (hockey players, cyclists, skiers, track and field athletes, sweemers, rowers and gymnasts) and found no difference in ACE genotype and allele frequency compared with controls. Similarly, the cohort examined by Karjalainen, Kujala, Stolt, Mantysaari, Viitasalo, Kainulainen et al. (1999) of 80 elite endurance athletes from Finnish national teams and one of the largest (192 athletes) studies by Rankinen, Wolphart, Simoneau, Maier-Lenz, Rauramaa, Rivera et al. (2000) which also included skiers, long and middle distance runners, cyclists and biathlon, was also result in failure to demonstrate an association between elite athletes and the ACE genotype. The possible mechanism of the effect of the ACE genotype on skeletal muscles probably occurs at the cellular level, and perhaps through the influence of the angiotensin II on the redirection of blood flow from type I muscle fibres to type II muscle fibres, which is a favoured process in power performance. The greater local production of angiotensin II will then increase muscle contraction to the maximum (Rattigan, Dora, Tong, & Clark, 1996). The other potential mechanisms by means of which various levels of angiotensin II could influence human performance, are via its effect as the direct stimulator of cellular growth (both hypertrophic and hyperplastic), as well as the induction of various endogenous growth factors and the facilitation of the sympathetic transmission by the increase of the release of noradrenaline from peripheral sympathetic nerve terminals and the central nervous system (Jones & Woods, 2003; Saxena, 1992). Brown, Balis, Gandhi, and Adama (1998) also cite the degradation of bradykinin which can influence the effectiveness of skeletal muscles through the effect of bradykinin on muscle blood flow and substrate utilization.

Regardless of the controversial interpretation of ACE gene polymorphism, its effect on human performance is evident, but that future research will shed light on the molecular mechanisms of action.

Alpha-actinin 3 (ACTN3) are a family of actin-binding proteins, which play an important role in maintenance and regulation of the cytoskeleton. Two isoforms of the alpha-actinin found in the human body, alpha-actinin 2, can be found in all the skeletal muscle fibres (slow and fast) as well as in the cardiac muscle and the brain, while alpha actinin 3 is limited only to type 2, fast fibres in the skeletal muscle. North, Yang, Wattanasirichaigoon, Millis, Easteal, & Beggs (1999) identified polymorphism in the ACTN3 gene, known as R577X, which is contained in the conversion of the codon for arginines (R) in position 577 with a stop codon (X). This change has lead to two versions of the ACTN3 in the human body, the functional R-allele and the zero X-allele. The homozygote for the X allel (the XX genotype) results in the complete absence of the alpha actinin in the human body. The XX genotype frequency differs in the population of humans in a range of approximately 1% among Africans, approxi-mately 18% among Europeans and of around 25% among Asians (Mills, Yang, Weinberger, Vander-Woude, Beggs, Eastel et al., 2001). It has been estimated that around one billion people worldwide are deficient in alpha-actinin 3. Alpha-actinin 2 is similar to alpha-actinin 3 and on the basis of that similarity it was believed that alpha-actinin 3 is functionally redundant, which means that its flaw is replaced, in terms of function, by alpha actininin 2. Nevertheless, research has shown that the ACTN3 has an independent function, which indicates a separate evolution and different expressivity, so that the ACTN2 cannot completely compensate for the loss of ACTN3.

Numerous studies have indicated a positive association between R alleles and the capacity to form strong muscle contractions. On the other hand, the presence of the X allele can be a predisposition for greater endurance in physical activity. Using these assumptions as a starting point, Yang, MecArthur, Gulbin, Hahn, Beggs, Easteal et al. (2003) used a sample of 302 caucasian elite athletes involved in 14 different sports, and found a higher frequency of 577R alleles among sprint and strength athletes, while the athletes involved in endurance sports showed slightly higher frequencies of the XX genotype compared with controls. More and more often, the possible role of the ACTN3 gene in the determination of the distribution of the type of muscle tissue is mentioned. Yang's assumption that alpha-actinin 3

enhances the formation of fast muscle fibres was confirmed, but other means and interaction between alpha actinines with metabolic enzymes in the regulation of the distribution of muscle fibres were not excluded.

Despite the different results, we can conclude that the polymorphism of the ACTN3 gene has a confirmed biological effect on skeletal muscles, and thus, the loss of alpha-actinin 3 from fast muscle fibers has a detrimental effect on sprint and power activities. The different percentage of the mutation of this gene has indicated that the natural selection in the evolution of man has had a balancing effect. In some cases, speed is preferable, and in others, endurance, which is why both types of the gene are present in the population.

Myostatin gene – Myostatin is a protein, a member of the TGFβ superfamily (the transforming growth factor beta). This protein is made up of two identical subunits, each of which contains 110 amino acids, in their inactive form, and for its activation we need a protease, which replaces the NH3 group with the more active COOH group. The gene which codes myostatin is marked as MSTN or GDF-8 (the factor of growth and differentiation – 8). Myostatin is primarily produced in the skeletal muscle cells, circulates through the blood and lymph and affects muscle tissue, slowing down the development of muscle stem cells. It has been proven to inhibit kinase which causes muscle hypertrophy through the synthesis of proteins. Thus, myostatin acts in two ways: inhibits muscle differentiation and inhibits protein synthesis.

This protein is active in the skeletal muscles prior to and after birth, and imposes a normal limit on muscle growth. The mutation which reduces the production of functional myostatin leads to an increase in muscle tissue. People suffering from this mutation of the homozygote have significantly increased muscle mass, while those with a mutation of the heterozygote have increased muscle mass to a slightly lesser exent.

The confirmation of the connection between myostatin and the size of the skeletal muscles has launched an entire series of questions about the role of myostatin as a potential means for the increase of the overall performance in sport. The increase in muscle mass also means an increase in overall strength, and it is considered that the inhibition of myostatin can significantly increase physical ability, despite the fact that an increased growth of muscle cells carries with it the risk of the reduction of the overall strength based on the cell and histological changes in the muscle cells.

Future research will certainly determine the role of myostatin in the human body in more detail, as well as its influence after birth and in adult human tissue, the interaction with other growth factors and its role in the regeneration of tissue after injury.

The insulin-like growth factor I (IGF-I) – is a protein which is important for skeletal and muscle development. It is a mediator of the growth hormone and affects cells through the receptors of the tyrosine kinase, but it can also bind with an insulin receptors. The activity of the IGF-1 includes: the stimulation of cell growth, the activation of the protein synthesis and glucose and glycogen uptake (Barton-Davis, Shoturma, & Sweeney, 1999), the inhibition of degradation of muscle proteins, regeneration of tissues, the modulation of the immunological response, affecting the synthesis of cytokines and immune system cells (Humbel, 1990).

Several mutations on this gene are known to affect an increase in muscle mass, and a significant connection between the IGF-1 genotype and the increase in dynamic strength has also been determined (Musaro, McCullagh, Paul, Houghton, Dobrowonly, Molinaro et al., 2001; Sweeney, 2004). The role of the IGF-1 is also very significant in muscle damage and reparation processes, where it affects the activation of satellite cells and proliferation, which then connect muscle fibres and lead to their regeneration (Engert, Berglund, & Rosenthal, 1996). Several growth factors including insulin-like growth factor (IGF-1) and transforming growth factors (TGF-β1 and β2) enhance and accelerate the normal bone regeneration pathways (Landesberg, Roy, & Glickman, 2000).

The vascular endothelial growth factor (VEGF) – The gene that codes this factor can affect the formation of new blood vessels. Vascularization is determined by means of blood flow, and not average use. In the case of increased physical activity, the need for blood flow is increased six to eight times in comparison to blood flow during rest. By creating VEGF in the muscles, the necessary vascularization is increased, and in that way the muscles get the necessary nutritive substrates and the oxygen necessary for muscle contraction.

By the use of this factor in athletes, by means of better supply of oxygen and nutritiens to the tissue, a fatigue can be delayed to a significant extent, and the production of energy can be improved and metabolite production decreased.

PPAR – delta (Peroxisome proliferator-activated receptor - delta) – is a nuclear protein receptor, which functions as transcription factors for the

regulation of gene expression in adipose tissue, the heart and muscles, placenta and the like. Studies carried out on animals have shown that the PPAR-delta plays an important role in the metabolic adaptation of many tissues to the changes in the outside environment, and which take part in the regulation of the metabolism of fatty acids in skeletal muscles and adipose tissue through the expression of genes involved in the takeover of fatty acids, beta-oxydation, the creation of energy and the formation of "slow"muscle fibres (Evans, Barish, & Wang, 2004). These results open numerous questions regarding the manipulation and solutions for complex physiological abilities such as fatigue and endurance.

Erythropoietin (EPO) – is a glycoprotein hormone secreted in the liver and kidneys. It induces erytropoiesis through the stimulation of receptors on the stem cells of bone marrow, which guide their differentiation into erythrocytes.

Patients who suffer from severe anemia, cancer patients after chemotherapy or patients with kidney failure, may benefit tremendously from therapy of EPO, because their bodies produce inadequate amounts of erythrocytes.

For the past ten years, synthetic version of EPO is often used by athletes for increasing the oxygen carrying capacity of the blood. But, increasing the number of erythrocytes, EPO increases the risk of hypertension, heart attack or stroke. As the athlete becomes dehydrated during training, blood volume is reduced, which increases the possibility of thrombosis.

GENES USED FOR TREATING SPORTS INJURIES

Gene therapy can be succesffuly used to treat sports injuries, which include muscle injuries, ligament and tendon ruptures, cartilage lesions, bone fractures and the like. By transfering genes which code the corresponding growth factors into the injured tissue, tissue regeneration can be improved. Considering the fact that bone development includes the activation of several genes in the differentiation of the osteoblast from mesenchymal precursor cells (IGF, FGF, transforming growth factor β, VEGF), the application of these genes has, in the animal models, shown a significant improvement in the speed of fracture healing (Southwood, Frisbie, Kawcak, & McIwraith, 2004). A number of these factors (VEGF) and bone morphogenic protein, activate osteoblasts improving the rate of bone repair. Thus delivery of the DNA encoding such growth factors will enhance repair. Although this is allowed as a therapeutic tool, it happen that expression of these factors increase the strength of bones and tendons, so that giving an adventage to competitors. Therefore, although these treatments are promising, the clinical application is limited.

Insufficient data has been collected on gene transfer in ligament, tendon and cartilage treatments, since what we are dealing with is another type of tissue, but these procedures should soon become a part of clinical practice. VEGF (vascular endothelial growth factor) is expressed in ruptured and foetal human Achilles tendons, but not in normal adult Achilles tendons (Pufe, Petersen, Tillman, & Mentlein, 2001).

Muscle injuries constitute between 10 and 55% of all injuries sustained by athletes. Local ingestion of IGF-1, basic fibroplast growth factor (bFGF), or nerve growth factor (NGF) after injury increases the number and size of regenerating myofibers in different animal studies (Hoffmann & Gross, 2009; Skuk & Tremblay, 2008). Such strategies should be applied to patients suffering from muscular diseases but not to athletes to speed up a healing process because there are many more risks than potential benefits.

Nitric oxide (NO) is a fundamental participant in the basic biology of sports related injury. This molecule plays a role in the physiology of articular cartilage, tendon ligament, muscle, bone, intervertebral disc and synovium. Gene therapy techniques may allow alteration of local NO concentrations. Ongoing studies are beginning to provide answers on many questions and may allow for the eventual clinical use of gene therapy in the treatment of musculo-skeletal injury (Huard, Li, Peng, & Fu, 2003).

One important aspect of regenerative medicine is tissue engineering. It includes three main elements: cells, factors or stimuli (growth factors, cytokines) and biomaterials (Service, 2008). The biomaterials for tissue engineering, which can be derived from natural (like collagen), or synthetic sources (polymers of lactic and glycolic acid), need to be biodegradable and biocompatibile. Due to the vascularization, healing of bone often precedes readily, but the soft tissues like cartilage, tendons and ligaments are poorly vascularized and heal slowly. Stem cells present an ideal and promising option for tissue engineering of tendons (Sharma & Maffulli, 2008). Mesenchymal stem cells (MSCs) are capable of undergoing differentiation into a variety of specialized mesenchymal tissues, including bone, tendon, cartilage, muscle, ligament, fat and marrow stroma (Caplan & Bruder, 2001; Sharma & Maffulli, 2006). Another potential application of MSCs is ex vivo, de novo tissue engineering. This technique

involves construction of whole body tissues in the laboratory, and their inplantation into patients. Several studies have demonstrated engineering whole tendons in such a manner (Calve, Dennis, Kosnik, Baar, Grosh, & Arruda, 2004; Cao, Liu, Wei, Xu, Cui, & Cao, 2006).

These strategies are currently at an early stage of development and their full impact needs to be the focus of intense research.

DETECTION OF GENE DOPING

Gene doping is difficult to detection using standard doping tests. The proteins transferred by means of genes are human in origin and do not differ from the rest of the endogenous constituents. Blood and urin tests are not suitable, because the recombinant protein is expressed locally and thus a biopsy is necessary for tissue sampling. The use of protein markers, as indicators of any disturbances in normal physiology is a possible solution, with a previous individual analysis (screening) of the set of proteins under physiological conditions, and during the study phase, the use of microchip technology and DNA barcodes is necessary.

Given the many different genes that are used in doping, it is necessary to know their expression and metabolic pathway. Since 2000 it has been possible to identify recombinant EPO. The electrophoretic mobility technique provides a direct measurement of urine levels and it is based on the principles that the rHuEPO molecule is less negatively charged than the endogeneous EPO molecule. It may also be possible to detect minute traces of gene transfer vectors using highly sensitive polymerase chain reaction - based techniques such as single-copy primer-internal intron-spanning PCR procedure described by Beiter, Zimmermann, Fragaso, Armeanu, Lauer, Bitzer et al. (2008). Another approach is to use indirect technique to demonstrate potential gene doping by looking for the consequence of the genetic manipulation such as changes in patterns of target gene expression (trancriptomics), proteins (proteomics) or their metabolites (metabalomics).

Affinity-based biosensors (ABBs) would be suited for application of strategies involving the detection of gene or delivery vector DNA sequence, recombinant protein product, or other indirect biomarkers. ABB based on use of oligonucleotide probes specific for the endogene-ous sequence. It can be used to detect the recombinant protein by utilizing specific antibodies (Minunni, Scarano, & Mascini, 2008).

ETHICAL ASPECTS OF THE USING GENE IN SPORT

In accordance to the principles of the World Health Organization, in medical ethics, adhering to two principles is of primary importance: respect for man and loyalty to the ideal of humanity. But gene therapy abuse in sport dissolves these very principles of humanity. The creation of a super athlete is in direct opposition to the basic ethical principles, endangers the health of the athlete, and the very spirit of sport could be disrupted. Fair play includes equality of circumstance and mutual respect, as well as the satisfaction of victory, while every other type of victory would be a form of cheating of nature and humanity. It is necessary to make a clear line of division between therapy, and help sick and vulnerable on the one hand, and abuse on the other, at the same time passing judgment on it, in a legal, moral and ethical sense.

CONCLUSION

One of the most important aspects of the development of genetics is the possibility of successful gene therapy. We now have the ability to treat many genetic and non-genetic disorders, which were only until recently untreatable. However, it is necessary to overcome many difficulties in its application, until it is fully incorporated in clinical practice. The viruses that transfer genes attack and destroy the immune system. Once introduced, a gene cannot be extracted, and the reactions of the body could be negative, as the introduction of a foreign gene might cause insertion mutagenesis. These are just some of the problems being faced in gene therapy application.

Even though we believe that gene therapy in the treatment of sport injuries is more effective than conventional methods, its application is still limited and has not been studied in full detail. *There is a fine line between gene therapy and gene doping in athletes*. A number of growth factors will enhance repair, but it happen the expression of these factors increase the strength of bones and tendons, so that giving an adventage to competitors. Therefore, although these treatments are promising, the clinical application is limited.

At the moment there are no evidence that gene doping is being practiced by athletes, but there is a concern that it will be used in the near future. First of all, it is necessary to meet athletes with the consequences of gene doping, provide to them clear information and present potential risks. What is most

concerned is the abuse of the therapeutic aspects of gene therapy, its application to the germinative cells and application in the field of sports for performance enhancement. The coordination of efforts by international scientific and sports communities to develop successful detection strategies are essential to overcome that problem. Victory and glory are strong arguments, but the health of young people, respect for fundamental and ethical principles, humanity, fair play game have a more lasting value and represent the heavier weight on the scales.

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