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Review article

USE OF BANNED EFFORT ENHANCERS AND GENE TRANSFER

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Abstract

Anabolic steroids, which facilitate the achievement of the grand prize, not only increase the competition and training threshold, but also trigger substance (growth hormone, insulin-like growth hormone-1, testosterone, etc.) addiction. Long-term use of these types of anabolic steroids can cause fatal diseases such as cancer. It is known that an average of 50% of athletes in some countries do not test positive in doping tests for their anabolic steroid use. This means that some of the athletes who have received Olympic medals so far have been clean in the tests, while some are registered as doping. Gene tests can be used to test claims that a genetic mutation is responsible for a positive doping test and to monitor gene doping and detect genetic markers. There are hundreds of gene variables that trigger athletic success, and the accelerated development of genetic therapy or treatment is increasing the possibility of gene doping. WADA aimed to prevent gene transfer or modifications by banning gene doping, which is the last point of unfair competition, in 2003. The probability of gene doping to be performed and to have a significant effect on the results of the competition is very low. Technology is advancing rapidly, but until we see the results of positive clinical trials that treat human diseases using gene therapy, it is unlikely that gene doping that has been attempted in under-the-ladder labs will be successfully implemented. The next century is the stepping voices of a new multi-gene and multi-factor beginning and a new world order in which digital life and molecular genetics take part together.

Keywords: drugs, doping, genetic tests, CRISPR Cas9.

1. Introduction

The World Anti-Doping Agency (WADA) has declared the application of prohibited ingredients and techniques that increase sportsmen and sportswomen efforts with extreme acceleration and disrupt quality life as doping or the use of prohibited substances, as a result of the checks of the selected individuals who reflect their individual efforts on the field or the podium in the international arena, at the end of the competitions or at the addresses they have been in at certain periods, In this context, some athletes who uses anabolic androgenic steroids, other anabolic agents, growth factors, hormones, elements that change blood structure or fluidity, gene and cell doping, etc., at every risk due to their effort-increasing and suppressive effects, methods and substances, will result in triggering factors at every risk due to their effort-increasing and suppressive effects, especially the unbearable attraction of the reward. Although WADA prohibits the use of the mentioned substances outside of competitions or inside the competitions due to their doping effect, in some countries these products and methods are allowed to be used without restrictions, even with the support of the authorities (worth mentioning the Icarus documentary shared on the internet is very engrossing and equally worrying) or completely turning a blind eye. Usage rates of performance altering substance are gaining momentum because of these situations. (1, 2). The fact that many of the banned drugs on WADA's list can be purchased from pharmacies does not change the fact that the use of these drugs will adversely affect human health. These types of drugs or specially prepared drug regimens are for people with serious health problems, not for normal and healthy individuals. Even natural products that are considered to be healthy (e.g. amino acids, protein powders, energy drinks, etc.) may contain objectionable substances. It is obvious that all drugs used have side effects in one way or another as because if substances that are not needed by the organism are used, it is inevitable to

face serious side effects. According to, results of the data obtained from the results of the studies conducted within the framework of the relationship between gene and athletic performance, it is an indisputable fact that individuals' different gene sequences resulting from inheritance and variations will cause the body's reactions to exercise stimuli of different types and intensities will not be similar (such as ACE and ACTN3 polymorphisms) and that each individual can only make an effort within their genetic limits. Inevitable end stemmed from sudden deaths or facing chronic diseases as a result of the gradual loss of their effects on the altered body functions, is the last point where the desire to move to the next stage by tearing or exceeding these limits with effort-enhancing substances is finalized. Inevitable end stemmed from sudden deaths or facing chronic diseases as a result of the gradual loss of their effects on the altered body functions, is the last point where the desire to move to the next stage by tearing or exceeding these limits with effort-enhancing substances is finalized. Anabolic steroids, which facilitate the achievement of the grand prize, not only increase the competition and training threshold, but also trigger substance (growth hormone, insulin-like growth hormone-1, testosterone, etc.) addiction. Long-term use of these types of anabolic steroids can cause fatal diseases such as cancer.

Research show that it is possible for some selected people to come out clean from the doping tests they have entered, despite the banned drugs they use due to their genetic makeup. It is known that the banned substance testosterone used by some people (10% of European descent) to grow their muscles did not appear in doping tests. Anti-doping tests that detect the use of testosterone are carried out by measuring the ratio of epitestosterone (E) to testosterone (T) (T / E), metabolites of testosterone glucuronide produced by the body and epitestosterone glucuronide. If the ratio of doping samples (T / E) taken from athletes before or after the competition exceeds ¼, they are directed to the next test regarding whether they use testosterone. If the athlete's test results are positive, he will be banned from the competition. If the sample taken from the athlete does not exceed this rate, the athlete is clean. In scientific research, it has been observed that athletes have different amounts of steroid residues in their urine due to differences arising from heredity. Similarly, it is known that because of the genetic mutation (MC1R) pain thresholds of redheads are quite high due to the mutation of the same gene (3, 4, 5).

Ethnicity has been observed to affect the urinary testosterone glucuronide epitestosterone glucuronide (T/E) ratios among athletes. Uridine diphospho-glucuronosyltransferase 2B17 (UGT2B17) is the most active enzyme in testosterone glucuronidation (6). According to studies, it has been stated that people who do not have the 2B17 gene after taking testosterone have a much lower T / E ratio than those who have at least one copy of the gene (7). When compared with multiple variables such as age, gender, circadian rhythm, or diet that directly affect steroid profile, genetic modification of the UGT2B17 gene is by far the most effective. UGT2B17 would be an effective strategy for detecting testosterone abuse (8). The gene in question (athlete gene) "uridine diphosphote-glucuronosyl transferase 2B17" variable can affect whether the results obtained in doping rooms are positive or negative. Selected athletes, even if they use the same dosage of testosterone as others, can take part in the rostrum like athletes who do not use any banned substances, without being stuck in the doping tests. The only difference between them is due to changes (insertion / deletion) in their genes. It is known that an average of 50% of athletes in some countries do not test positive in doping tests for their anabolic steroid use. This means that some of the athletes who have received Olympic medals so far have been clean in the tests, while some are registered as doping. In this case, it is likely that some countries consider genetic advantages in their selection of athletes. Because the selected people in question can overcome the sonars of doping tests despite using performance enhancing drugs (3, 4, 5). It is also quite thought-provoking that some Olympic athletes admit that they have been successful using doping years later. It is also possible that genetic test identification purposes can be used directly for anti-doping testing in the coming years. Gene tests can be used to test claims that a genetic mutation is responsible for a positive doping test and to monitor gene doping and detect genetic markers. Due to the deletion of the data obtained after the genetic tests, there is no cause for concern about genetic testing in the fight against doping in sports (9). Strict measures taken by WADA in recent years and banning some countries from competitions for a long time have significantly reduced the use

of performance enhancing substances. In this process, the outstanding performances in many sports branches without external support draw attention of the many. Especially despite his young age, Swedish athlete Armand Duplantis, who broke the world record in pole vaulting (6.17cm), was able to open a place for himself in the arena of champions before he turned twenty (9, 10).

2. Use of prohibited substances

The genetic advantages of the chosen people allow them to show a high level of effort. In addition to the geography, routine daily life, and motivation for the development of the peak effort at the maximum level, the correct sequence of the nucleotides (genetic markers) in the DNA helix also triggers the climb to the peak. The inherited characteristics are highly effective on muscle fiber type, anaerobic threshold, lung capacity, and aerobic threshold (9, 10). Aerobic power, the main determinant of long-term efforts, is largely genetically coded. Endurance athletes create the energy needed for muscle contraction and recovery by using the oxygen carried to the tissues in their muscles sufficiently and efficiently. Exercise efficiency or maximum oxygen usage capacity (MaxVO2) can be increased by training for a long time, but the rate of increase in MaxVO2 is determined by genetic structure. Many endurance athletes who went beyond their limits by unnaturally triggering the peak effort line increase disappointed many sports fans who followed them, when they had to admit years later that they used the hormone EPO to accelerate the oxygen to the muscles (3). Despite this, it is also thought-provoking that no banned substance was found in the samples.

3. Gene doping

With the development of science, some athletes started to get to know different methods and pharmacological agents, how and for what drugs they can use, by browsing websites to accelerate their physical fitness, muscle strength and athletic abilities. Gene doping has been expressed as "non-therapeutic use of modulation of cells, genes, genetic elements or gene expression capable of enhancing athletic performance" according to the banned substances list published by WADA in 2008 (11). There are hundreds of gene variables that trigger athletic success, and the accelerated development of genetic therapy or treatment is increasing the possibility of gene doping. WADA aimed to prevent gene transfer or modifications by banning gene doping, which is the last point of unfair competition, in 2003.

The purpose of gene doping is not to cure or prevent diseases, but to raise the talent line of the elected to the top. Gene doping is an ideal method for chosen ones who are attracted to reward (12, 13, 14, 15). Regulation of blood oxygen carrying capacity with EPO or VEGF as gene doping can provide a clear advantage in disciplines with dominant aerobic character. Similarly, genes that increase muscle strength and contraction speed are markers that can alter muscle utilization capacity depending on targeted activity (ACE, ACTN3, PPAR genes and mTOR activity, etc.) by promoting muscular hypertrophy (IGF-1 uptake). In addition, muscular injuries, pain and recovery times also limit athletes' athletic performance levels. Therefore, genes that produce analgesic endorphins are also possible candidates for gene doping (16).

Doping tests can detect prohibited substances in compounds manufactured by the body or cultured outside the body in urine or blood tests (12, 13, 14, 15). However, difficulties are encountered in processes such as determining multi-gene and multi-factor parameters and revealing changes in gene doping detection. The utopian trial method that can be used to check gene doping has not yet been determined. The insufficiency of tests to check gene doping is related to the fact that the protein coded by the gene transferred from the outside or by hereditory supervise cells will be structurally and functionally very similar to endogenous proteins. Since transgenic proteins, which increase muscle strength and muscle contraction speed, are produced locally in the injected muscle, it is not possible to detect them in samples. The best useful technique, muscle biopsy, is also difficult to use in athletes (11).

4. Potential candidate genes

Potential candidate genes are performance enhancement genes. Growth hormone (GH), insulin-like growth factor (IGF-1), myostatin (MSTN) gene, which affect the cardiovascular system by increasing the number of red blood cells in the bone marrow, vascular endothelial growth factor (VEGF), They are genes that increase muscle phenotype modifiers (PPARD, PGC1A) and eliminated soreness (12, 13, 14, 15). The erythropoietin gene is a glycoprotein hormone that enhancement the rate of erythrocytes generation in the bone marrow. For gene doping, EPO is used in the athlete's metabolizm by accesing a viral vector, thereby increasing the production of hemoglobin in the blood and oxygen transfer to the tissues. Growth hormone (GH) increases lipolysis, protein synthesis, muscle hypertrophy and strength by playing a role in stimulating glycogenolysis and increasing glucose release from the liver (11). Muscle pain is a warning that should not be ignored. Pain relievers or suppressants can allow sportsmen to exert the highest effort as long as possible. Another variant to these banned drugs is analgesic peptides such as endorphins and enkephalins. Endorphin, which prevents the decrease in lactic acid production or the increase of its threshold, is used to relieve pain and fatigue. It has been shown that in laboratory animals, the genes producing the peptides in question have an effect on the perception of inflammatory pain. Vascular endothelial growth factor (VEGF) supports the development of new blood vessels, allowing greater amounts of oxygen to be transported to the tissues. IGF1 is a factor that increases the rate of muscle growth (hypertrophy), triggered by growth hormone. An increase in muscle mass was observed in mice injected with IGF1. Combining IGF1 with multi-gene and multi-factor traits or muscle strength training programs can guide to even quick responses in muscle building (17). It has been reported that the PPARGC1A gene triggered after acute aerobic exercise controls glucose and lipid metabolism, and the effort level of slow-twitch muscle fibers causes an increase in aerobic and muscular endurance (18). The Myostatin (MSTN) gene has an inverse relationship with IGF-1, one of which inhibits muscle growth while another triggers it. A rare AA genetic variation of the MSTN gene causes the complete loss of MSTN gene function and the complete absence of the MSTN protein. Viruses can transfer genetic material to target DNA, allowing the gene of interest to reproduce its characteristics. The rare AA genetic variation of the MSTN gene can be inserted into the DNA in the nucleus of the target cell with gene carrier. These procedures, which have been successfully performed in recent years, have become a relatively safe system by reducing the possibility of an endogenous virus transmission (12, 13, 14, 15). The probability of gene doping to be performed and to have a significant effect on the results of the competition is very low. Technology is advancing rapidly, but until we see the results of positive clinical trials that treat human diseases using gene therapy, it is unlikely that gene doping that has been attempted in under-the-ladder labs will be successfully implemented. Will it be a process in which all kinds of risks are taken in order to be successful in the coming years? Otherwise, doubts about whether it will be the beginning of a new era in which different mutants (whose genes are changed) that emerged with the widespread use of CRISPR Cas9 technology took over the peak (15, 19). The answers to this question may be found in the incredible change provided by the CRISPR / Cas9 method.

Gene doping has emerged from gene therapy trials based on strategies to treat genetic diseases by modulating the activity of a missing gene or an existing gene. It can be performed in vivo (intracellular) of the target (artificial) gene into the human genome, by biological (viral vectors), physical (direct injection using an injector or gene gun) or chemical methods (using phospholipid vesicles known as liposomes). This method is administered directly to tissues or blood by means of viruses. The in-vitro (extracellular) method involves the transfer of genes from the person to the cells in the culture and the retransfer of genetically modified cells to the host. Gene therapy or doping using viral vectors (retrovirus, adenovirus, lentivirus) is the most effective method in this context (20). Gene doping increases the potential of strength and endurance abilities. In-vitro gene doping is the reproduction of cells taken from athletes by tissue culture and re-injection into the body or by gene transfer through viruses. Performance enhancing genes are transported to the cells through the blood by injecting DNA-containing viruses into the athlete (such as the transfer of growth hormone-producing genes to viruses in the laboratory and then injected into the body). Gene transfer can increase muscle strength and

endurance by affecting the energy use mechanism, affecting the diameter of the muscles, the speed and amount of blood flow to the muscles (12, 13, 14, 15).
CRISPR / Cas9 technology is preparing humanity for a new future. It shouldn't be too hard to predict what might change in the future. Scientists have discovered thousands of genes that cause disease, such as the FABP4 gene, and when you uncover how genes work, it's easier for you to control them. However, changing the gene in living cells is not an easy method. Yet, with the CRISPR / Cas9 technology, it has become very easy to make any particular changes. The ability to edit the DNA of all kinds of living things, even including humans, can be achieved by this method. CRISPR system is a method developed inspired by bacteria and with the discovery of this technology begins with the discovery of an anti-viral protection mechanism possessed by bacteria. When bacteria identify virus-derived DNA to protect against diseases from viruses, it produces two types of short complementary RNA against this DNA. The region identified for cutting and marked with RNAs combines with the Cas9 enzyme found in the nucleus (nucleus). Thus, the target viral genome (nucleic acids that make up the genetic structure of viruses consist of just one of the DNA or RNA and store the genetic information to be transferred to the host cell to produce nucleic acid) by shearing the virus out. Scientists have long been questioning whether they can take not only the infected DNA but any DNA, or rather change the structure of DNAs. It is probable that recently DNAs could be cut and replaced by other DNAs, and the process could be performed not only in test tubes but also in living cells. (15, 19, 21, 22). It has been shown that CRISPR / Cas9 can be easily used to edit genes in mammalian embryos such as rodents and monkeys. These embryos can then be implanted into guard animals and live-born animals with changes in their DNA can be produced. The first study showing that CRISPR / Cas9 can be used to manipulate genes in early-stage human embryos was published in early 2015. These experiments clearly show that genome editing can be studied in human genomes (21, 22). Scientists can put the copy of the DNA they want to edit into the cells living in the system. With the technology, the researchers were able to destroy the HIV virus from laboratory mice. Many diseases can be cured with this method. Genetic changes made by previous methods could not be passed on to the next generations, but the changes made with the CRISPR method can be transferred to the next generations. With this method, the structure of the genome we have soon can be changed, and what is in our favor is the possibility of living longer and healthier without getting sick (15, 19). The use of CRISPR will increase with the genetic basis of diseases and solving the functions of genes. With CRISPR, the health status, well-being, and quality of life of patients will increase. With the improvement of the quality of life and environmental conditions, people will want more. The number of demands such as being longer, smarter, stronger, resistant to X disease (such as the COVID 19 virus) will gradually increase. This will lead to differences in the usage area of CRISPR before preimplantation. There may also be a tendency towards not only treatment but also artificial selection (21, 22).

5. Gene doping risks and side effects

There are many challenges and risks associated with gene therapy and gene doping. Although it is relatively easy to send genes to a specific cell type in a laboratory setting, targeting the right cells in the right tissues to give athlete strong muscles are an extremely difficult process. Even in a highly controlled laboratory environment, only some cells will efficiently take up the gene delivery vector and incorporate newly inserted genes into their genomes (genetic codes found in inheritance material). Efficiency will drop even more if you consider a whole organism. Most adult adolescents or genetically modified animals used in scientific research are born with genetic modifications or are modified in embryo state and consist of a single or only a few cells. The challenges of genetic changes are also related to the size of the animal species to be altered. Although viral vectors (transport of genetic material into the cell) are easy to genetically modify in a mouse, making them in larger creatures such as humans raises the problem of producing enough viral vector. The efficiency of generating a viral vector containing the genetic material is quite poor, and the majority of delivered vectors are likely to be non-functional. Besides the uncertainty of the effects of genetic modification, gene doping also

comes with serious health risks such as cancer and potential immune responses. Cancer can develop if a genetic change mistakenly turns on the cancer gene or knocked out a cancer-suppressing gene (12, 13, 14, 15). The physiological side effects of EPO use are primarily the accelerated increase in hematocrit values, which may increase the likelihood of stroke, myocardial infarction, thrombosis, and an increase in total peripheral vascular resistance. Like insulin administration, IGF1 can cause profound hypoglycemia. Evidence of the health risks associated with GH use can be listed as insulin resistance, impaired glucose tolerance, and limited effectiveness of the cardiovascular and respiratory systems. Side effects of GH gene doping are also associated with intracranial hypertension, headache, peripheral edema, carpal tunnel syndrome, joint and muscle pain, or cardiomegaly. Suppression of the myostatin gene or protein has been shown to trigger the growth of muscle tissue in terms of cell number and size. In some pathological conditions, an increase in muscle mass over a short period of time can promote hypertonic cardiomyopathy, resulting in a heart attack. Excessive growth of muscle mass also leads to overload of the musculoskeletal system, increasing susceptibility to bone and tendon injuries (11).

6. Conclusion

It is not yet known what effects the changes made in the human organism through gene transfer will have on the structure of DNA, so it is thought that it will not be possible to use such technologies for now. Unlike previous methods, many genes acting in conjunction with CRISPR technology can be used to target many genes at the same time. This method is a great advantage for curing complex human diseases caused by a single mutation. However, it is very difficult to realize whether a wrong arrangement was made when making such changes, so it seems quite difficult to predict what benefits or not mutational changes will bring to humanity in the future (15, 19). Genes encoded by evolution over millions of years have survived and developed their own defense systems by fighting all kinds of viruses that have emerged to date. However, as in the Covid virus (although it is associated with the Type I interferon gene, which increases the risk of the disease and prevents the production of seventeen proteins, there are still discussions about whether it is produced in the laboratory environment (23). The next century is the stepping voices of a new multi-gene and multi-factor beginning and a new world order in which digital life and molecular genetics take part together.

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