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Current threats on gene doping - a systematic review

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Abstract

Introduction: The publication of the first results regarding the effects of gene therapy on muscle mass and muscle force in rodents has sparked sustained interest from the part of trainers, athletes and other categories of specialists concerning this ethically-questionable revolutionary method, which would increase sporting performance. The *purpose* of this study is to find out if gene doping poses a real threat in today's performance sport through synthesizing the main information regarding the ways of using and the screening of the ways in which it has been used by athletes, as well as the measures undertaken in later years in connection to their regulation. The *methods* employed were the perusal and analysis of information published in research papers (accessed through Clarivate Analytics and Google Scholar) or in other official sources by using the following key words: genetic doping coupled with screening, effects, WADA etc. The *results* have highlighted the fact that, from the multitude of information obtained, a significant part is based on assumptions and discussions without any palpable evidence; regardless, one may also encounter some papers supported by objective data which made possible the extraction of real and coherent information. *Conclusions:* Gene doping remains the latest challenge in the doping matter and it raised the interest of athletes and trainers in order to try to control and manipulate performance parameters and processes such as muscular mass, strength, power, speed, endurance, tissue regeneration and repair, pain perception; the steps that have been made recently for the screening and regulation of gene doping are consistent and ensure a relatively safe environment for clean sport.

***Keywords**: gene doping, effects, detection, athletes, WADA*

Rezumat

Introducere: Publicarea primelor rezultate privind efectele terapiei genice asupra masei şi forţei musculare la rozătoare a declanşat un interes deosebit din partea antrenorilor, sportivilor şi a altor categorii de specialişti pentru această metodă revoluţionară, dar nu tocmai etică, care ar putea creşte performanţa sportivă. *Scopul* acestui studiu a fost acela de a afla în ce măsură dopingul genetic este o ameninţare reală în sportul de performanţă al zilelor noastre prin sintetizarea principalelor informaţii privind posibilităţile de utilizare şi depistare a folosirii acestuia de către sportivi, precum şi măsurile care s-au luat în ultimii ani în ceea ce priveşte reglementarea acestora. *Metodele* utilizate au fost căutarea, analiza şi sintetizarea informaţiilor publicate în articole ştiinţifice (accesate prin Clarivate Analytics şi Google Scholar) sau în alte surse oficiale folosind următoarele cuvinte cheie: doping genetic în asociere cu depistare, efecte, WADA etc. *Rezultatele* au evidenţiat faptul că, din multitudinea de informaţii găsite, o mare parte se bazează pe presupuneri şi discuţii nefondate pe dovezi concrete; totuşi, există şi articole susţinute de date obiective care au făcut posibilă extragerea unor informaţii reale şi coerente. *Conclusions:* Dopingul genetic rămâne una dintre provocările actuale în doping şi a crescut interesul sportivilor şi antrenorilor în scopul controlului şi manipulării unor parametri şi procese specifici performanţei sportive cum ar fi masa şi forţa musculară, puterea, viteza, anduranţa, regenerarea şi repararea tisulară, percepţia durerii; the steps that have been made recently for the screening and regulation of gene doping are consistent and ensure a relatively safe environment for clean sport.

***Cuvinte cheie**: doping genetic, efecte, depistare, sportivi, WADA*

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Introduction

Even though the concept of *gene therapy* appeared for the first time in the 60s and 70s, this treatment was applied only in 1990 to a 4-year-old girl suffering from a genetic disorder – deaminase deficiency, which profoundly affected her immune system. Later, in 1998, a group of researchers from the University of Pennsylvania School of Medicine published a paper in *Proceedings of National Academy of Sciences USA* that confirmed the use of a common virus for the insertion of a gene into the DNA of muscle cells of young and old mice in order to produce insulin-like growth factor 1 (IGF1). They obtained an increased muscle mass and strength by approximately 15% in young mice and reversed age-related muscle changes in old mice, making them 27% stronger than they were before. The publication of the data regarding the production of the so-called "Schwarzenegger mouse", as the press called it, triggered immense interest from the part of athletes and trainers, as well as, obviously, as was expected, from the part of the factors involved in the fight against doping [1]. Within the same time frame, other studies were published that were conducted on rodents and which demonstrated the effects of gene therapy on the skeletal muscle [2].

As happened with the occasion of the discovery and use of other substances or methods of doping, initially, the desire to increase sporting performance overcame the fear of potential side effects on the athletes' organism; even in this case, almost instantaneously, the desire for athletes to undertake genetic "interventions" has appeared, without knowing the negative side of their use at the time [1,3]. Besides the fact that it could trigger persistent changes in muscle mass, it appears that this type of approach would be difficult to screen [1,3].

At present, in the Prohibited List published by the World Anti-Doping Agency (WADA) at the beginning of each year, within the category of Methods, together with *M1 - Manipulation of blood and blood components*, *M2 - Chemical and physical manipulation*, one may also find category *M3 – Gene doping* [4].

Obviously, gene doping appeared as a concept due to the development of gene therapy; still, there is a huge difference between the two: if, in the case of gene therapy, the recipients are animals / people in whose case the treatment of certain pathological conditions is attempted (as is the case of certain genetic disorders - haemophilia, thalassemia, certain immune deficiencies, severe ocular diseases, or acquired ones - various forms of cancer, Parkinson's disease, degenerative neuro-muscular diseases etc. [5-9]), in the case of gene doping one aims to increase athletes' performance, thus in the case of perfectly healthy human beings [10].

The purpose of this study is the answer the following questions: what degree of proximity really exists between gene doping and athletes today and how can one effectively fight it?

Material and Methods

The search for research papers that contain data regarding gene doping was made through the following methods:

- Accessing the Web of Science (WoS)– Core Collection (Clarivate Analytics) database through the search topic "gene doping" for all the documents that were indexed in the 1975 – 2018 timeframe (up to 31 October 2018) [11];
- Accessing the Google Scholar database and the selection of papers from 2015 – 2018 with the same topic; it should be mentioned that, due to the high number of information present here, I have chosen only the last four years as a timeframe for this database, taking into account that the recent papers may bring valuable information as regards the proposed analysis, and for the old ones, I have considered only the WoS content as relevant.
- Accessing the official website of WADA [12].

After highlighting the searched documents through the employment of the *gene doping* topic for the 3 databases, criteria of inclusion and exclusion from the study have been applied.

The criteria of inclusion for the research papers were the following:

- To be written in English;
- To explicitly contain the key term "gene doping";
- To be full-text accessible or at least at an abstract level (through the institutional profile on Enformation, the ANELIS PLUS 2020 platform);

The criteria of exclusion referred to:

- The documents that contained “gene doping” only tangentially, without being a key term of the study;
- The documents that contained inconclusive or presumptive information regarding the topic of the study;
- The documents that appeared in two or in all three initial selections have been considered only once.

Results

By following the search protocol, with the subsequent application of the above inclusion and exclusion criteria, we found:

- on Web of Science – Core Collection: 1680 papers, from which 110 were selected for analysis;
- on Google Scholar: 575 papers, from which 43 documents were selected for analysis, considered relevant for this study;
- on the WADA website: 81 papers/announcements linked to gene doping; from these, 34 were selected.

After these criteria were applied, the final selection and the analysis of the information from the 187 documents was conducted. To support this study, 63 papers/documents were selected for a qualitative analysis.

The selection process is represented in Figure 1.

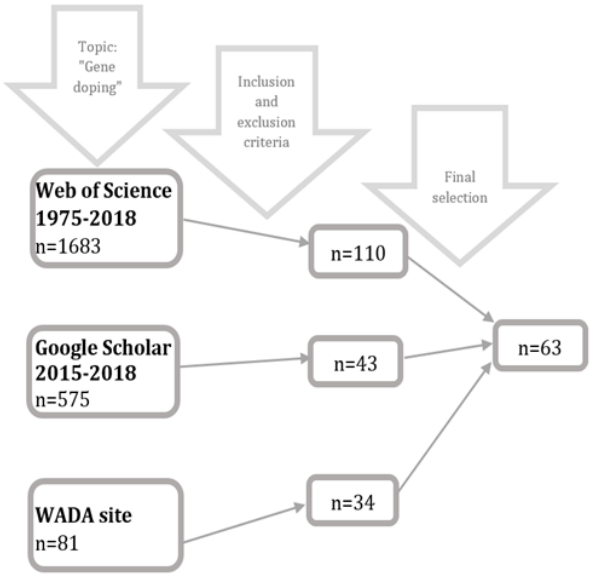

Figure 1. The selection process of the documents for systematic review

Table I. WADA's Prohibited List changes regarding Gene doping (M3)[4]

Year of changes	The text regarding Gene Doping (M3 on Prohibited List)
2004	"Gene or cell doping is defined as the non-therapeutic use of genes, genetic."(p. 6)
2005	"The non-therapeutic use of cells, genes, genetic elements, or of the modulation of gene expression, having the capacity to enhance athletic performance, is prohibited."(p. 5)
2009	"The transfer of cells or genetic elements or the use of cells, genetic elements or pharmacological agents to modulating expression of endogenous genes having the capacity to enhance athletic performance, is prohibited. Peroxisome Proliferator Activated Receptor δ (PPARδ) agonists (e.g. GW 1516) and PPARδ-AMP-activated protein kinase (AMPK) axis agonists (e.g. AICAR) are prohibited." (p. 6)
2010	"The following, with the potential to enhance athletic performance, are prohibited: 1- The transfer of cells or genetic elements (e.g. DNA, RNA); 2- The use of pharmacological or biological agents that alter gene expression. Peroxisome Proliferator Activated Receptor δ (PPARδ) agonists (e.g. GW 1516) and PPARδ-AMP-activated protein kinase (AMPK) axis agonists (e.g. AICAR) are prohibited."(p. 6)
2011	"The following, with the potential to enhance sport performance, are prohibited: 1. The transfer of nucleic acids or nucleic acid sequences; 2. The use of normal or genetically modified cells; 3. The use of agents that directly or indirectly affect functions known to influence performance by altering gene expression. For example, Peroxisome Proliferator Activated Receptor δ (PPARδ) agonists (e.g. GW 1516) and PPARδ-AMP-activated protein kinase (AMPK) axis agonists (e.g. AICAR) are prohibited."(p. 6)
2012	"The following, with the potential to enhance sport performance, are prohibited: 1. The transfer of nucleic acids or nucleic acid sequences; 2. The use of normal or genetically modified cells." (p. 6)
2013	"The following, with the potential to enhance sport performance, are prohibited: 1. The transfer of polymers of nucleic acids or nucleic acid analogues; 2. The use of normal or genetically modified cells." (p. 6)
2018	"The following, with the potential to enhance sport performance, are prohibited: 1. The use of polymers of nucleic acids or nucleic acid analogues. 2. The use of gene editing agents designed to alter genome sequences and/or the transcriptional or epigenetic regulation of gene expression. 3. The use of normal or genetically modified cells."(p. 6)

WADA Regulations

As was the case of any new method or substance for increasing sportive performance, gene doping also underwent a period of latency, until the authorities involved in the fight against doping have regulated their use. The current interpretation of gene doping in the Prohibited List was introduced for the first time in January 2018 [13], after WADA declared in 2003 for the first time that "the nontherapeutic use of cells, genes, genetic elements or the modulation of gene expression, having the capacity to enhance athletic performance, is prohibited", and, in 2004, gene doping first appeared as a method in the Prohibited List. Beginning with 2004, the chapter regarding Gene Doping from the Prohibited List has continuously been subjected to changes and completions. These are depicted in Table 1 [4].

Moreover, in 2004, WADA created an Expert Group on gene doping. The Expert Group's task is to study the latest advances in the field of gene therapy, the methods for detecting doping; WADA also financed a series of projects with these purposes [12].

"Opportunities"

Within this category, so-labelled in a pejorative way, I have synthesized the data with regard to the specific reasons for which an athlete and/or trainer would consider gene doping; they are mainly connected to the increase in muscle mass, speed, strength, improving endurance and energetic metabolism, increasing the pain-perception threshold or the acceleration of tissue healing and regeneration.

The increase of endurance has been obtained through the targeted expression of an activated form of peroxisome proliferator-activated receptor delta (PPAR-δ), which transformed fast fibres (type II) towards a slower and more oxidative phenotype (type I) [14]. Moreover, these changes confer resistance to obesity with improved metabolic profiles (even in the absence of exercise), and a higher insulin sensitivity [15,16].

Injection of a plasmid with a vascular endothelial growth factor (VEGFA) gene into the muscle of patients with chronic critical limb ischaemia led to improved distal flow [17, 18]; as a consequence, the gene could increase endurance through an increased intake of oxygen at the muscular level. For the same reason, the erythropoietin (EPO) gene expression may be increased by gene doping and followed by a bigger production of red blood cells [19].

The hypoxia-inducible factors (HIF-1) gene encodes proteins involved in the process of hypoxia, angiogenesis and erythropoiesis activation or in the regulation of glucose metabolism. The genes controlled by the HIFs include those coding for proteins that stimulate red cell production, as well as those encoding glycolytic enzymes which produce additional energy in conditions of relative hypoxia, which are crucial in the attempt to achieve improved aerobic athletic performances [17, 19].

The activator of AMP-dependent kinase (AMPK), also known as AICAR, is an analogue of adenosine monophosphate (AMP); AICAR may reduce the level of anabolic processes, including synthesis of fatty acids and proteins and may increase the level of catabolic pathways such as glycolysis and fatty acid oxidation [20,21]; also, it was demonstrated that after 4 weeks of AICAR administration to mice subjected to training, their speed and strength increased by 20-40% [22].

The increase of muscle mass and force through genetic manipulations has also been the subject of significant research and publication; thus, the introduction of the insulin-like growth factor-1 (IGF-1) gene in rats led to an increase in muscle mass and strength and increase in endurance [23]. IGF-1 stimulates cellular proliferation, somatic growth and differentiation [10]. The inducement of growth hormone (GH) production may be involved in gene doping, especially by considering that it has an anabolic effect on muscle mass, it stimulates lipolysis and, at the same time, *the processes of tissue mending* at the level of the locomotor apparatus, thus hastening their healing [10, 24]. On the other hand, the suppression of the gene that produces Myostatin – MSTN (a protein that inhibits muscle growth) in mice has been achieved, which had as an effect the augmentation of muscle mass and force [25].

The increase in speed and muscle force was also achieved in mice by appealing to the transfer of the phosphoenolpyruvate carboxykinase (PCK1) gene – an important link in the Krebs cycle, thus obtaining an improvement of the body composition through the stimulation of lipolysis and gluconeogenesis [26, 27].

Gene therapy may also be used to stimulate the production of endogenous opioids and,

consequently, to increase *the threshold of nociception*. There are studies that have confirmed the increase of the secretion of endorphins and enkephalins in animals with acute or chronic lesions; for these reasons, the method could stir the interest of athletes to diminish their perception of pain and alleviate their nociceptive behaviours [28-30]. Those who would crave such a change are, possibly, athletes with acute or chronic pathologies accompanied by pain or those who practice contact sports.

Threats

The main threat as far as gene doping is concerned is linked to the health of the athlete, above that of ethics or any other type; the adverse effects that gene doping may have on the athletes' organism have not been probed at present in the case of gene doping; however, these may be deduced or extrapolated by knowing the side effects of gene therapy applied to human subjects up to the present moment or from those described in the studies conducted on animals. According to Wells, the threats induced by gene doping are determined by two elements: 1. the product and/or the procedure that induces gene change; thus, the employment of adenoviral vectors was associated with illnesses or even deaths [31]; 2. the uncontrolled expression of the gene in the organism [10].

Thus, it has been demonstrated that HGH and IGF-1 increase the risk of oncogenesis [32], and the overexpression of hypoxia-inducible factor 1(HIF-1) and other angiogenic factors may determine an increase in vascularization, which may be involved in the formation and development of solid tumours; these factors are also responsible for: intracranial hypertension, abnormal vision, headache, nausea, vomiting, peripheral oedema, carpal tunnel syndrome, pain in the joints and muscles, overgrowth of the cartilage of the nose and jaw, cardiomyopathy, insulin resistance and diabetes [17, 33].

The overexpression of EPO is associated with an increase in haematocrit, which may enhance the likelihood of stroke, myocardial infarction, thrombosis and an increase in total peripheral vascular resistance [34]; moreover, the EPO gene transfer has caused autoimmune anaemia in macaques [35,36]; ruptures of tendons and ligaments, bone disorders (in the case of the suppression of the MSTN gene) have been noted following the massive development of muscle mass, in the detriment of the other components of the locomotor apparatus [17].

Even if the adverse effects of gene therapy are presently monitored through rigorous controlled studies, those of gene doping do not benefit from the same care; that is why the potential adverse effects are significantly difficult to highlight, report and, eventually, treat. Even though there are voices who claim that gene doping is not an immediate threat [37], a study published in 2011 regarding the detection of non-approved therapeutics categorized as anabolic and gene doping agents in products distributed via the Internet (concerning AICAR, GW1516 - a PPARδ receptor agonist, and a selective androgen receptor modulator - SARM) revealed that athletes could buy them from the Internet suppliers in a relatively easy way; even though they were labelled "for research only" and the main substances were considerably lower than indicated on the label, the potential doping possibility and its side effects still exist [38].

Detection of gene doping

In the last 15 years, the approach of each Olympiad or major international competition brings to the fore the threat posed by genetic doping.

WADA has allocated important resources in the last decade to find new ways of screening genetic doping; on the organization's website, one can find 23 financed research projects that aim to find effective ways of screening genetic doping; among those targeted, the list is topped by EPO, IGF1, VEGFA, GH, HIFs, PPARD, PCK1, MSTN, and some of their recombinant protein products (rEPO, rhGH) [1, 2, 12, 13].

The methods used in anti-doping laboratories are based on the direct identification of new substances or metabolites and on the indirect evaluation of genetic or protean changes or of metabolic patterns induced by doping [39].

According to Friedmann T., the chair of WADA's Gene Doping Expert Group, the screening of gene doping should depart from the idea that exposure to a doping agent changes the expression of some genes in the organism and, consequently, one should target the biological responses to that agent, the genetic

"signatures" of a specific drug (with special reference to EPO and HGH). Furthermore, he forecasts the employment of methods to screen viral vectors used in gene transfer [40].

On the other hand, a response from the other side, that of the "cheaters", should be expected, as far as gene doping is concerned, by the other way around – to obtain false negative results in anti-doping tests.

A certainty as far as gene doping is concerned does exist; a gene doping test was developed by researchers at the National Measurement Institute in Sydney, Australia, rolled out for the Rio Olympics tests (in 2016) for sequencing differences between endogenous and synthetic versions of the EPO gene [41].

Discussions

The research papers that were initially selected from the WoS (n=1683) may be placed in a multitude of fields, which highlights the fact that gene therapy and its unwelcome descendant, gene doping, are subjects of interest for researchers from the following fields: biochemistry, molecular biology, pharmacology, biotechnology, medicine, biophysics, biomaterials, nanoscience and nanotechnology [11]. Thus, gene doping is, from this point of view, a complex, multi-, inter- and transdisciplinary issue.

At present, studies have been dedicated to more genes with a major potential of being used in the treatment of pathologies that could have a severe prognosis without this approach; a part of these genes may become subject to gene doping; the analysed data suggests that, from a longer list, IGF1, GH, MSTN and rhGH may play a major role in strength sports, while EPO, VEGFA, HIF-1, PPARD, PCK1 and rEPO are essential in endurance sports [17].

Gene therapy has progressed enormously in recent years, but 2017 was considered a key year in this field; extraordinary results have been obtained in the treatment of severe illnesses (sickle-cell anaemia, lymphoma, bone marrow cancer, epidermolysis bullosa, retinal diseases, haemophilia [42]. This progress will indirectly impact the potential of gene doping because the mechanisms of action, the methods of gene therapy and their side effects have become better known.

Without knowing the intimate mechanisms of gene therapy or gene doping, I set myself alongside those who highlight the uncertainties and the potential problems induced by the attempts to screen gene doping: is it possible that the changes to the structure of an athlete's DNA through doping will be so difficult to screen that they may be confused with a configuration that may exist naturally? If the changes are present only in a part of the body (for example, in a certain muscle area – decisive for the increase of sportive performance), is it possible for them to evade screening? [43] Will one always know where the border between gene therapy and gene doping resides, given that certain athletes would require, in a certain context, such a therapeutic approach (without an alternative)? Will gene therapy ever become a therapeutic use exemption (TUE)? We might also rhetorically ask: how equal are we as far as our native genetic baggage is concerned?

On the other hand, the advanced studies in the field of human genetics could be of real help to performance sports in an honest way [44]; the awareness to the "gene profile" required by a certain sport or its branches could immensely improve the selection at every level of future champions through an awareness of "genetic aptitudes" (given by the genetic configuration that each of us have from our intrauterine life); of course, besides these assets, great performance requires many other factors, but it should be desired that the "start" (referring to genetic baggage) be as close as possible to the ideal.

Even though there is a clear official regulation regarding gene doping and there are also methods of screening certain changes induced in this way, one may still encounter the vanity of certain athletes and/or trainers etc., financial interests (which cannot be ignored in certain situations) that open doors to gene doping, doors which lead to a yet-unclear place.

Despite the evidence that some people are interested in attempting gene doping [38, 45], the following painstaking question remains unanswered: if no people have been screened as having appealed to gene doping, is this because they did not resort to it or that they have not been screened yet? Perhaps the answer will surface in Tokyo in 2020.

Conclusions

In the last 30 years, the progress in the knowledge of the human genome, of gene therapy and in the connected fields has been immense; it is similar and directly linked to the progress in technology.

Even though the innovations recorded as far as the manipulation and employment of genes and cells "with the potential to enhance sport performance" are mentioned and updated in the Prohibited List, they still pose the capacity of troubling us and they truly represent a "threat to the integrity of sport and the health of athletes"[12].

Gene doping remains the latest challenge in the doping matter; the latest progress in this field has raised the interest of athletes and trainers in order to try to control and manipulate performance parameters and processes such as muscular mass, strength, power, speed, endurance, recovery and rehabilitation (tissue regeneration and repair), pain perception.

On the other hand, the steps recently taken to screen and regulate gene doping are consistent and they ensure a relatively safe environment for a clean sport.

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