THE GENE DOPING: RECENT APPROACHES

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DOPAJUL GENETIC: ABORDĂRI RECENTE

Așa cum este definit de WADA, dopajul genetic este „utilizarea non-terapeutică a celulelor, genelor, elementelor genetice sau modulatorilor de expresie a genelor care au capacitatea de a îmbunătăți performanța atletică”.În ultimii ani, editarea genomului a avansat ca modalitate terapeutică, în principal în studiile clinice și dezvoltarea produselor, aprobate de FDA și EMA.Prin urmare, terapia genică ar putea fi utilizată cu ușurință în scopuri necorespunzătoare, cum ar fi dopajul genetic. Există câteva studii primare axate pe metode bazate pe secvențiere de noua generație[Next generation sequencing NGS] pentru a detecta editarea genelor la sportivi.

As defined by WADA, gene doping is "*the non-therapeutic use of cells, genes, genetic elements or modulators of gene expression that have the ability to enhance athletic performance*".

There are some molecular tools, like micro-seeding gene therapy, cationic liposomes, macromolecular conjugate, and gene-activated matrixes, that can be used to deliver a modified version of a gene, but the use of viral vectors is the most effective means for gene delivery. Retroviruses and adeno-associated viruses have been the most successful vectors in the clinical setting [1; 2]. These viruses are an excellent strategy to modify cells: they are programmed to transfect the cells by inserting their genetic material inside the cell and integrating it into the host genome, and they multiply by using resources from the host cells. Moreover, they have low immunogenicity, are a stable support for gene expression, and can be tissue-specific [3;4]we developed PCR protocols allowing the detection of very small amounts of transgenic DNA in genomic DNA samples to screen for six prime candidate genes. Our detection strategy was verified in a mouse model, giving positive signals from minute amounts (μ20 l. Nevertheless, these viruses are attenuated, and their replicating activity is controlled during gene therapy.

For the gene editing there are some techniques used: zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and particularly the CRISPR and CRISPR-Cas9. These tools are mainly employed to edit genes in somatic and germline cells of different species.

Furthermore, these technologies are being used to edit human somatic cells with a view to therapies that can be applied in healthcare, especially in the field of cancer immunotherapy through modified T cells [5;6].

In the last years, genome editing has advanced as a therapeutic modality, mainly in clinical trials and product development approved by the FDA and EMA. For example, the FDA has approved clinical trials that use the ZFN technique for in vivo insertion of therapeutic genes in hepatocytes for hemophilia B, mucopolysaccharidosis I, and mucopolysaccharidosis II [5;6]. RNA interference by naturally occurring miRNAs or synthetic small interfering RNAs (produced synthetically) is another method of gene expression regulation that has contributed to gene therapy [7].

However, gene therapy could easily be used for improper purposes such as gene doping [8]. Non-therapeutic genetic manipulation in sports, like the use of normal or genetically modified cells and transfer of nucleic acids or their analogues, is considered gene doping.

Nowadays there is a set of genes included at the close attention: erythropoietin encoded gene (EPO), myostatin blockers, insulin-like growth factor (IGF-1), growth hormone (GH), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), leptin, endorphins, enkephalins, α actinin 3 (ACTN3), cytosolic phosphoenolpyruvate carboxykinase (PEPKC), and peroxisome proliferator-activated receptor-δ (PPARδ) and many others (more than 100 potential targets) [9;10].

There is no WADA standardized method to detect gene doping. For a detection technique to be approved, it must fulfil extremely stringent criteria established by the WADA [11]. One of the techniques that is most frequently employed to identify therapeutic vectors and transgenic expression is real-time PCR, which allows both the identification of extraneous DNA sequences (e.g. vector sequences or copy number variations of a candidate gene), as well as quantitative and qualitative measurements of mRNA transcribed by a candidate gene. A promising new and highly specific, efficient, and rapid method to amplify DNA is the loop-mediated isothermal amplification (LAMP). Furthermore, it can analyze dry blood spot and can be easily applied in the competition venues, thereby accelerating the process of identifying doping in athletes [12].

In the case of the myostatin gene, a study with rats provided very interesting results: liquid chromatography coupled to high-resolution mass spectrometry (LCHRMS) helped to identify the introduced genetic material (small interfering RNA – siRNA) in urine samples after a single intravenous administration. In some cases of gene doping involving a gene introduced into a specific target tissue, testing for doping is difficult because biopsy, an invasive technique, would be necessary, which would

be a considerable limitation of the detection method. Image analysis is an alternative to invasive detection methods and are already being used in gene therapy research for gliomas. When it comes to detecting gene doping in humans, two image methods stand out for clinical use: positron emission tomography (PET) and single-photon emission computed tomography (SPECT) [13;14]. These techniques are more sensitive, can monitor the gene expression, and avoid some complications like invasive procedures and immunogenic reactions in humans.

Biomarkers (which represent a possible measurement parameter that is altered due to an individual intervention in a system) and gene expression markers are promising alternatives to detect gene doping through *omics* approaches. The use of transcriptomics and proteomics to discover biomarkers may be based on existing physiological and biochemical knowledge, but genetic analytical techniques can also be employed to recognize differences between people that have been submitted to gene doping or not. An example is the study to detect recombinant human erythropoietin (rHuEpo) biomarkers in human whole blood: this study used transcriptomics analyses to identify a predominant signature of altered erythropoiesis in the users at injection doses ranging from microdoses [15]the World Anti-Doping Agency (WADA to high doses [16].

There are some primary studies focused on NGS-based methods to detect gene editing within athletes[16].But PCR-based methods for gene doping detection report a sensitivity of about 4–14 copies of gene doping copyDNA in 1000 ng whole-blood-isolated human gDNA. Current NGS-based methods reach a sensitivity of 1296 copies in 1000 ng gDNA, which is ~100-times lower than PCR-based methods.The method can further be optimized to increase the sensitivity by increasing the percentage of copyDNA fragments in the captured library [16].One way to do this is to isolate DNA (copyDNA and gDNA) from blood plasma instead of whole blood, similar to what we have done for noninvasive prenatal testing.The method can further be optimized to increase the sensitivity by increasing the percentage of copyDNA fragments in the captured library. The percentage of gDNA compared with copyDNA in plasma is far lower than that in whole blood because of the removal of white blood cells. Alternatively, the capturing efficiency could be increase by specific blocking of gDNA sequences during the capturing process with nonbiotinylated probes. These future adjustments will improve sensitivity and lower the costs since fewer reads are needed to detect each gene doping copyDNA transcript.

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IMPACTUL RELEVANȚEI RELAȚIEI MULTIFORME DINTRE SĂNĂTATE ȘI CONSUMUL DE CARNE DE PASĂRE

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IMPACT OF THE RELEVANCE OF THE MULTIFORM RELATIONSHIP BETWEEN HEALTH AND CONSUMPTION OF POULTRY MEAT

The relevance of the relationship between health and consumption of poultry meat is multifaceted, and must be analyzed in detail, with specific scientific attention, associated with a balanced ration and the health of the population. The poultry meat has multiple priorities – variable energy, unsaturated lipids, highly digestible proteins, B-group vitamins, minerals and other valuable components. The poultry meat is considered the optimal protein component in the ration, which is associated with reducing the incidence of risk of overweight and obesity, cardiovascular disease, diabetes et al. Also, poultry meat, and white in particular, especially, in the composition of the ration contributes to the general physiological favoring of the body in various states and conditions (conception, pregnancy, growth, senescence) and provides the body's needs in calories and protein.