Editorial

Drug Testing and Analysis

Received: 1 August 2011 Revised: 3 August 2011 Accepted: 4 August 2011 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/dta.353

Misuse potential of molecular biology and biotechnology in sport

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Keywords: gene doping; misuse potential; molecular biology; sport

The topic of gene-doping initially arose in the 1990s, almost at the end of the human genome project, which completed in 2000. At this time, gene therapy started to show its first promising results.

Gene therapy was first successfully applied in 1990 in the treatment of a four-year-old child suffering from a severe combined auto-immuno deficiency (SCAD).[1] A number of further successes were followed by a heavy setback in 1999 when a young man died as a consequence of a gene therapy.[2] Presently gene therapy is once more on its way to becoming a normal therapeutic measure where conventional therapies fail. The increasing practice of gene therapy of course also implies the risk of misuse in sport – gene doping. *Gene doping in sports - fact or fiction*? and *The gene manipulated high-level athlete – a horror vision*? were questions raised in 1998 in Germany and preliminary answers were given by experts and scientific symposia in the following years.

The World Anti-Doping Agency, founded in 1999, organized its first scientific workshop on gene doping in 2002 in New York. Further conferences in Stockholm (2005) and St Petersburg (2008) followed and various national and international meetings have been dedicated to the specific issue of misusing knowledge arising from biotechnological advances in medicinal research.

In 2010, The Federal Institute of Sports Science of Germany organized its second conference on gene doping to assess the potential danger of misuse of modern methods of molecular biology. It featured three special areas of genetics with a view to elucidating both the possibilities of misuse and its detection: genome, transcriptome and epigenetics.

Polymorphism at the myostatin gene locus and EPO-receptor gene locus are of particular interest when aiming at muscle growth and elevated oxygen capacity, respectively. Many other gene loci are related to performance in sport but are not as unilocular as gene loci previously enumerated. Results of family studies and other comparative investigations show various participating genes but significance is rarely given and 'lucky punches' of significance of relatively small samples lose their ground when the investigated population grows larger.

Polymorphism of the gene locus UGT2B has been shown to influence the metabolism and elimination of testosterone and epitestosterone and their concentration in urine. Consequently, the impact of this phenomenon on modern sports drug testing, a field that considers the steroid profile as one of the most important sources of information in doping controls, was elucidated.[3]

Further, research concerning the role of mitochondrial DNA (regarding the energy cycle and mitochondrial myopathies related to defects of mtDNA) was presented in the context of gene therapy; its misuse potential in sport, however, has been implausible so far.

The genome is not an isolated double strand of DNA in the nucleus but is encapsulated by histones, a group of protecting proteins surrounding the DNA forming strings of spheres. In a figurative sense, these shields are cut at distinct places as the first step of transcribing a part of DNA, for example, by so-called zinc-finger proteins. Evidence or indication of misuse in sports has not (yet) been provided; methylation and demethylation of DNA, as another epigenetic regulatory mechanism of gene expression, may offer a tool of manipulation.

The transcriptome is a mirror of genetic activity and is essential for transporting genetic information. The mRNA generated in the nucleus consists of exons and space-filling introns; the latter are removed immediately by enzymes in the cytoplasm so that the active mRNA is composed of exons only. This offers one option to detect the misuse of gene therapy because only RNA can be copied to cDNA in the cytoplasm for inserting this cDNA with a gene ferry (e.g. a virus). The resulting gene then consists of exons only and can be identified as xenobiotic.[4]

Introns will also be digested to different forms of RNA which act as regulators of further translational processes. Although a lot of knowledge about these effects is at hand, they are not sufficiently calculable yet.

Manipulations on tissues also result in free circulating DNA (CNA) which might be useful for sports drug testing purposes. CNA today is a powerful diagnostic tool for various diseases, for example, different forms of cancer.

Samples for the detection of doping usually consist of urine and particularly blood. The latter is often preferred as genetic manipulation can be tissue-specific and not detectable in urine. Detection in blood might be possible but limited in respect to its specificity. One of the most likely target tissues of manipulation is the muscle. In order to determine whether muscle tissue has been the subject of gene doping, muscle biopsies are presumably the only way to obtain an adequate sample of cells for assaying. Although a microbiopsy may be a possibility to obtain a sufficient number of cells without injuring the muscle, which would imply a functional reduction, such specimens are currently not in the scope of anti-doping authorities.

In summary, gene doping still seems to be an emerging problem in high-level sports as gene therapy develops to become a controllable tool in medicine and in the manipulation of livestock

animals.[5] There are a lot of educated guesses but it appears impossible to predict if, where, and when gene doping will be determined for the first time.

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