Gene Doping in the Equine Athlete: The New Normal in the "Art of Cheating"?
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Image: Adobe Stock

The success of any racehorse is determined by genetics, training and nutrition, which ultimately impact physical traits such as speed, muscular strength and endurance. While training and nutrition no doubt contribute significantly to a racehorse's performance, inheritance plays an equally if not greater part in influencing performance. At least, that is what the racing industry has based its breeding programs and stud fees on since the birth of horse racing. And of course, doping as a means of performance enhancement, has gone hand in had with these principles in an attempt to manipulate those attributes.

With the development of sophisticated genetic technologies for the genes responsible for these traits comes the potential for novel therapies that will not only benefit treating diseases and musculoskeletal injuries but more insidiously, artificially enhance the performance of the racehorse, otherwise known as gene doping.

Gene doping in human athletics has been on the radar for almost two decades and the race to develop detection methods has been fraught with difficulty, although progress is being made on that front. The World Anti-Doping Agency (WADA) has defined gene doping as the "transfer of polymers of nucleic acids or nucleic acid analogues, or the use of normal or genetically modified cells that have the capacity to enhance athletic performance", and has been listed among banned substances and methods since 2003. *See* What is Prohibited | World Anti-Doping Agency (wada-ama.org).

As gene therapy has shown progress and positive results in recent years, it has made its way into doping regimes in the horse racing world and, just like pharmacological doping agents, it is a threat to both the health of the horse as well as the integrity of the racing industry and a fair wagering environment. Moreover, as with other illegal agents, neither the performance enhancing effects nor adverse side effects of this doping method are well known. Unfortunately, as is observed time and time again, the exploitation of new drugs and other doping agents that can be abused tends to be more rapid than the ability to develop test methods to detect them.

What is gene doping?

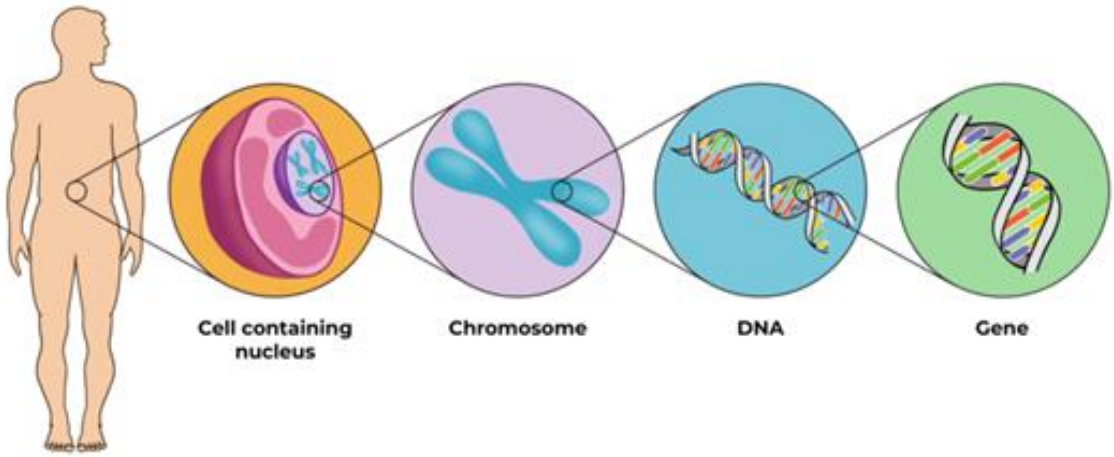
Gene doping is an off-shoot of human gene therapy. Gene therapy is used to modify or manipulate the expression of a gene or to alter the properties of biological cells for the purpose of restoring some function related to a damaged or missing gene to treat or cure a disease. Gene therapy aims to fight a disease by replacing, inactivating or repairing a disease-causing malfunctioning gene.

Therefore, gene doping can be defined as the non-therapeutic use of gene therapy in individuals who do not require it, with the intent of enhancing athletic performance. While this is morally unethical for obvious reasons, more importantly, gene doping could be dangerous, and perhaps even fatal.

In simple terms, gene therapy, and similarly gene doping, involves the introduction of genetic material into cells – essentially the introduction of specific genes that will, in the case of gene therapy, restore proper function to that gene, and in gene doping, enhance athletic performance.

The relationship between cells and genes

The body is composed of trillions of cells, the basic building blocks of all living things. Inside the nucleus of the cells, there are thousands of genes. These are comprised of a distinct sequence of "nucleotides" that form part of a chromosome. Genes carry the necessary genetic information and instructions for making proteins and enzymes, which help build and maintain the body (e.g. muscles, bones, blood).



Biology 101 – The Anatomy of Evolution

How are genes introduced into cells?

Gene therapy is not as simple as injecting genes into the bloodstream. Genes cannot enter the target cells on their own, so to put new pieces of DNA into cells in the body, it is typically packaged in a virus. These transport vehicles are called "vectors". Viruses have a natural ability to deliver genetic material into cells, however, before they can be used to carry the genes into cells they are modified to remove their ability to cause an infectious disease.

Some viruses, such as retroviruses, integrate their genetic material, including the new gene, into a chromosome while, adenoviruses introduce their DNA into the nucleus of the cell, but the not the

chromosome. By far the most common class of viruses used today are the viral vectors called "wild-type"adeno-associated viruses (AVV). These are not associated with any pathogenicity unlike some naturally occurring viruses, result in long-term gene expression, and can be engineered to target specific cell or tissue types. *See* Adeno Associated Virus (AAV) for Cell and Gene Therapy (addgene.org).

The vector containing the gene can be given intravenously directly into the target tissue in the body where it is then taken up by the individual cells (*in vivo*). Alternatively, the target cells can be removed from the body, the cells cultured in the lab, and genes inserted, then re-introduced to the body (*ex vivo*).

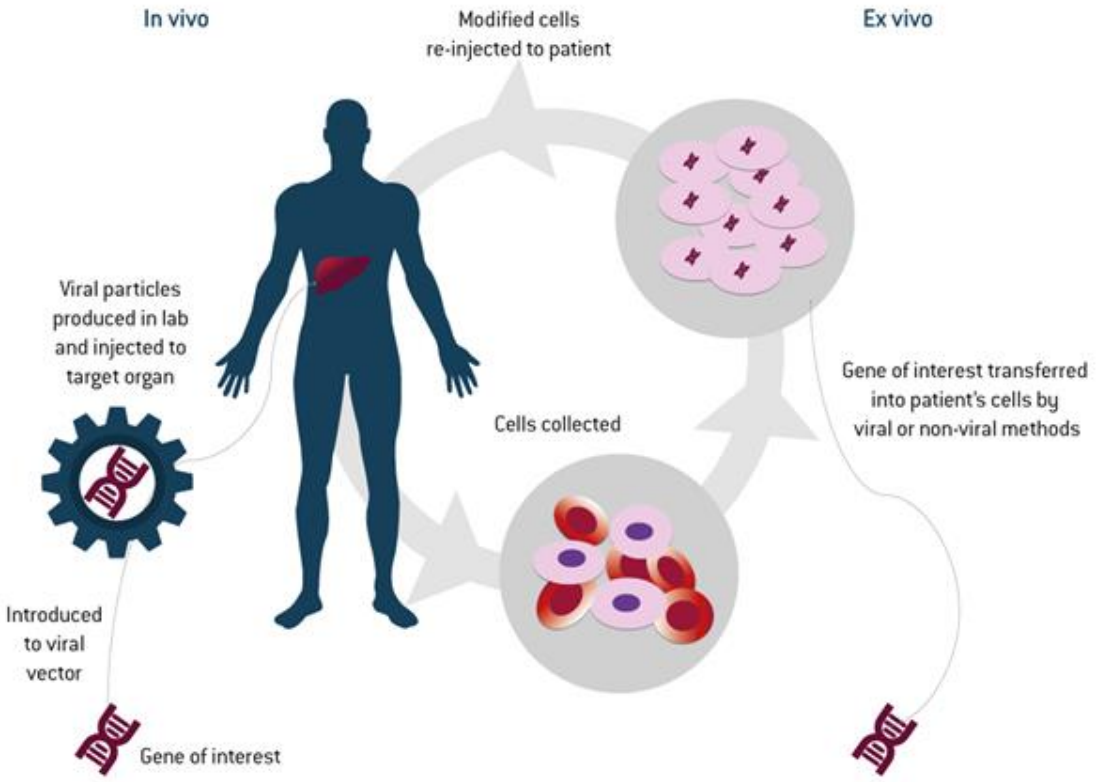


Image from An Introduction to Cell and Gene Therapy

The process is much more complex than described and there are different types of delivery vehicles other than viral vectors (e.g. plasmid DNA, bacterial vectors) as well as methods of gene editing (CRISPR) which are all employed as human gene therapy products. CRISPR technology is a subject all on its own and is both controversial and at the same time at the cutting edge of gene therapy for therapeutic purposes as well as gene doping in human athletes. *See* https://www.sportsintegrityinitiative.com/scientists-take-step-towards-gene-doping-detection/.

But for the purpose of this discussion, and brevity, will not be dealt with.

Gene doping in sports

Because genes are responsible for the manufacture of proteins and substances that affect cellular structure and function, and if that protein has been shown to be a function of an athlete's performance, it follows that more of that protein may further enhance performance. With the advent and progression of success in gene therapy treatments comes the inevitable application of its use in the sports world whether that be

human or animal. What better sport than horse racing, renowned for its nefarious doping abuse, to experiment on its charges? The future of gene therapy is no doubt exciting and beneficial, but the darker side is its potential for its abuse in sports, despite the potential risks. And indeed, in the last few years, it has become a growing concern in the horse racing world. *See* https://tuesdayshorse.wordpress.com/2019/07/04/gene-doping-of-racehorses/.

Equine performance genes for gene doping

In an effort to determine genes associated with racing performance, commercial DNA screening of horses began in 2000 with a racehorse genetic analysis company in the UK called Thoroughbred Genetics Limited. Six years later, the horse genome sequence was finalized, which allowed scientists to identify genome arrays responsible for equine disease and performance based on studies of human genetic pathways attributed to these features. Since this time, a patented genetic test, based on several of these genes, has been used to predict the performance potential of a racehorse over different distances. Genes known to have a favourable effect on a horse's speed, strength, ability to resist fatigue or mental aptitude are representative options for gene doping. These include; oxygen regulation genes, muscle response genes, energy metabolism genes, and pain and injury repair genes. *See* https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/full/10.1002/dta.2198.

There are several genes that have been recognized to improve athletic performance, some of which have been implicated in human sporting events. For example, the gene PPAR delta is a gene that was introduced to mice in an experiment (2004) that allowed them to increase their muscle mass that proved to increase their endurance. Insulin-like growth factor-1 (IGF-1) has also been investigated for its role in determining muscle mass and strength. Similarly, expression of the myostatin gene (*MSTN)* has a strong influence on skeletal muscle mass as do other transforming growth factor (TGF) genes. And then of course there is erythropoietin (EPO), the major regulator of red blood cell production, first made famous by Lance Armstrong. If the gene responsible for EPO production is introduced, this would allow the horse to increase its production or potency of EPO naturally through genetic modification and increase the blood's oxygen-carrying capacity. These are just a few of the possible mechanisms that could be exploited via genetic manipulation to enhance performance in the race horse. An in-depth scientific discussion of genes that could potentially be used in equine gene doping is described in the article, "Equine performance genes and the future of doping in horseracing". *See*https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/dta.2198.

What about the risks?

While all of this sounds like the doper's dream, gene therapy has been developed for the treatment of potentially fatal diseases and the repercussions are tolerated when it comes to a matter of life and death, but not so in the healthy athlete. When these techniques are used, human and horse alike, the narrative tells us that the potential side effects can outweigh the pursuit of success on the playing field. Viral vectors used in the transfer of genetic material can trigger a powerful immune response that can lead to multiple organ failure and death. It can also inadvertently create off-target effects where genes not intended for modification are affected and result in the disruption of normal cellular function. Moreover, over- or under-expression of genes encoding proteins can have calamitous effects. For example, the overexpression of EPO can dramatically increase blood viscosity and obstruct regular blood flow, while overexpression of myostatin (*MSTN* gene) can result in a rapid increase in muscle mass without allowing the heart to adapt, thereby elevating the risk of heart attack. See https://morgansl.com/en/latest/gene-doping-fiction-or-future. Even more sinister, if gene therapy in the horse is used on germ cells, which would alter gametes and be passed onto offspring (versus the somatic cells in tissue), this could lead to unpredicted effects in progenies.

These are just a limited few of the deleterious risks associated with gene therapy designed for therapeutic purposes by reputable sources. Now imagine the use of gene doping products produced and supplied by unregulated and unaccountable clandestine laboratories; the risks are even greater – Frankenstein's monster re-created?

Detection of gene doping in horses

Over the last several years, the idea that a horse's genes could be altered to enhance performance has grown from an entirely theoretical perspective to one of reality. Scientific advances in the field of gene therapy have evolved for the purpose of disease and injury intervention, but at the same time, offers a tempting alternative to drugs for improving athletic performance. These developments add to the corruption already present in the racing and breeding industries, and the need to have the capability to detect its presence. To avoid widespread use, tests must be developed to effectively avert the proliferation of gene doping as a means to cheat.

Detection of gene doping also presents an entirely new situation when it comes to routine testing. Unlike other drugs used for doping purposes, which exist as small molecule pharmaceuticals, gene doping vehicles activate cells to produce performance enhancing proteins. These are almost indistinguishable from naturally occurring proteins, which makes detection more difficult.

Recently however, researchers at the University of Pennsylvania School of Veterinary Medicine have developed a new test to precisely detect the local administration of gene doping agents in equine athletes. "The findings from the novel study, supported in part by the Pennsylvania Horse Breeders Association (PHBA) and the Pennsylvania Sate Horse Racing Commission, are a significant breakthrough in the collective fight to advance the welfare and integrity of sport for both horses and humans. " *See* https://phys.org/news/2021-03-presence-gene-doping-equines.html.

The study and team of researchers, led by Mary Robinson, Ph.D., VMD, DACVCP – assistant professor of Veterinary Pharmacology and Director of the Equine Pharmacology Laboratory at Penn Vet's New Bolton Center – developed and validated a quantitative real-time polymerization chain reaction test (PCR) that is able to detect the presence of a gene doping agent in both plasma and synovial fluid after injection into the joint. Not only does it provide a quick, convenient test for pre-race testing, it is also able to detect the agent in blood for up to 28 days. This is significant in that it represents a broad window of opportunity to detect illegal doping both in and outside of competition.

This is a game-changing development:

> "The science is closing in on those who seek to use these advancements for wrongful means; the more we learn with each study, the harder it will be for individuals who seek to cheat the system using gene doping strategies." *See* https://phys.org/news/2021-03-presence-gene-doping-equines.html.

This research is being conducted as part of a much larger New Bolton project that seeks to expand on its equine "BioBank". The BioBank is a database consisting of the analysis of numerous types of samples that identify biomarkers in equine athletes. The objective is to eventually create "biological passports" for sport horses that could be used both for predicting pre-existing injuries as well as detecting gene doping agents.

Currently, the PCR test is only capable of detecting a specific gene therapy, but a third study in progress aims to refine the test methodology such that multiple gene doping agents can be identified for even lengthier periods of time.

" We still have a lot of work to do to better understand the nature of bio-markers and how to fully harness their capabilities, but the science for detecting gene doping is getting there and much more quickly than any of us could have anticipated when we started this research," added Robinson. "Ideas that once may have seemed unattainable—like a hand-held, stall-side testing device—are now coming into sight as real and tangible possibilities. We just need continued support to help get us there."
See https://phys.org/news/2021-03-presence-gene-doping-equines.html.

Gene doping – today and beyond

With the advent of gene doping, horseracing is facing even more difficult challenges. As in the past, those that exploit illicit means to enhance performance, continue to seek novel methods that are both effective and difficult to detect. Gene doping is a perfect candidate. Over time, gene therapy has advanced in terms of both safety and efficacy, and with it the risk of its misuse as a doping agent in horseracing has become reality. So as to remain a step ahead of drug cheats and to maintain the integrity of the sport, PCR-based methods offer a viable prospect for detection in the equine athlete.

To date, several genes have been identified as having an effect on equine performance, and are thus potential contenders for gene doping. Moreover, with time, as genetic technologies in understanding their effects on equine performance evolves, the catalogue of potential doping genes will increase. Implementation of a detection method is therefore paramount to dissuade those contemplating the use of gene doping, as well as protecting the health and welfare of equine athletes.