

PROJECT REVIEW

“Informatics, Cross-Study Analysis and Molecular Signatures for the Detection of Doping.”

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The search for accurate and effective indicators for doping in sport requires the use of many different methods and tools. Traditionally, such tests have relied on the detection of suspected doping agents themselves. However, modern genetic methods have become available that promise not only to improve traditional methods to detect doping substances, but also to provide completely new methods to identify doping by detecting changes in the ways that genes are expressed in doped cells and tissues. WADA has established an extensive research program toward this aim and a number of WADA-supported laboratories have examined the effects of a variety of agents on gene expression such as anabolic steroids, growth and related factors and agents that affect oxygen delivery to tissues. However, detecting such changes is complicated by the fact that the various studies all use different materials and experimental methods.

To identify key changes that identify with scientific and legal certainty the effects of a doping substance and to distinguish useful signals from others caused by training, nutrition, ethnic background, gender, age, etc., it is important to compare the results from many independent studies by using the most powerful modern informatics and computational methods. We have established a WADA-supported informatics facility in this laboratory and have used it to obtain preliminary proof that simultaneous comparative analysis of several independent studies can tentatively identify genes or sets of genes that are altered by exposure to doping agents and that may provide new genetic signatures of doping. We now propose to collaborate with other WADA investigators to expand these initial comparative analyses to a larger set of independent WADA research studies and to published studies in the general biomedical literature. We will include studies related to growth factors and muscle function, oxygen delivery, anabolic steroids and modulators of gene expression.

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Result and Conclusion

PROJECT 10C10TF

TITLE: “INFORMATICS, CROSS-STUDY ANALYSIS AND MOLECULAR SIGNATURES FOR THE DETECTION OF DOPING”.

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TITLE: “COMPARATIVE TRANSCRIPTIONAL STUDIES OF HGH AND ERYTHROPOIETIN”.

These two related studies were designed to test the concept that exposure of humans to doping agents disturbs the normal expression of many of the 20,000 genes in human cells and whether those changes can be used as a rigorous proof or a “signature” of exposure to specific doping agents. These kinds of studies are made possible by modern genetic techniques that allow an estimate of the extent of expression of all human genes on a single square inch silicon chip. Our studies also were designed to determine if several studies carried out under different conditions in different laboratories could easily be analyzed even though they used slightly different techniques and methods of analysis. This method of analysis of multiple and slightly different sets of data from different studies is called “meta-analysis” and examples of meta-analysis have been successful in many other kinds of research. We therefore carried out meta-analysis of three independent studies of the effects on gene expression in blood samples from athletes of administration of human growth hormone (HGH) and three separate studies of the effects of erythropoietin (Epo) or hypoxia in the blood cells of human athletes and in mice.

As expected, we found that the genes that are expressed incorrectly after exposure to HGH and Epo are different from each other. However, we also discovered that the separate studies identified only a small number of genes that were disturbed in the same way in all three studies, leading to a conclusion that those genes are probably not specific “signatures” for exposure to HGH or Epo. We have concluded that these results could mean that these drugs do not cause significant changes in gene expression in blood cells. We prefer an explanation that the three separate HGH studies and the diverse Epo/hypoxia differed in many details that introduced too many slight differences in the timing of testing, dosages, methods of tissue preparation and methods of analysis that probably have hidden the gene expressions shared by the different studies. We are impressed that in other very recent studies supported by WADA in which one laboratory has carried out very careful studies of humans exposed to Epo, reproducible changes have been found that consistent with a genetic “signature” for exposure to Epo, demonstrating that extensive and well controlled single studies under some

conditions can be an effective approach to identifying the genetic changes in blood samples or other tissues. We propose that a reasonable next step is to carry out a similar study with HGH exposure and compare results from such a study with those obtained in our meta-analysis. We are confident the overall concept of genetic signatures of doping is a correct and robust one and will add important new tools to the prevention and detection of doping.