PROJECT REVIEW

"Detection of Growth Hormone Doping by Gene Expression Profiling of Peripheral Blood Cells in Humans"

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Current strategies for detecting growth hormone (GH) abuse in sport rely on quantifying GH-responsive proteins or measuring changes in the concentration of pituitary-derived GH isoforms in blood.

The aim of this project is to develop a test for GH doping based on the analysis of gene expression in peripheral blood cells. There is strong evidence that white blood cells respond directly to GH and indirectly through many anabolic and metabolic circulating factors. These secondary effects may continue long after the withdrawal of the GH stimulus and their gene profile characterisation could provide an extended time window for detecting GH use.

We propose to undertake this study by obtaining white blood cells from subjects currently enrolled in a WADA-funded GH administration study being undertaken to identify the most sensitive protein markers of GH in serum and the influence of testosterone on these markers. This proposal will not only enhance the scope and value of the existing study but will also allow comparison of biomarker and gene profiling approaches for diagnostic benchmarking.

The gene expression profiles will be measured using microarray technology and analysed by sophisticated statistical programs to identify a gene set which is a fingerprint of GH use. The gene set will be validated independently by quantitative PCR, a gold standard for measuring gene responses in cells. The gene set will be tested rigorously against placebo and other GH treated samples from the same study, and finally against an independent set with samples from subjects positive and

negative for GH excess or administration.
It is envisaged that this developmental work will lead to the design of a diagnostic gene chip that can be implemented for large scale diagnostic use.

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Results and Conclusions

Initial microarray analysis was undertaken using the Affymetrix platform. We have performed 98 Affymetrix Human U133 Plus 2.0 microarrays spanning four treatment groups and forty individual subjects. To determine the effects of treatment, we have compared an individual's baseline (week 0) and final treatment sample (week 8). Due to the small changes in gene expression, a second analysis was conducted using the Agilent Technologies platform, a more recently developed transcript profiling platform with enhanced sensitivity. The two colour methodology of the Agilent 44K G4112F array allows direct comparison of an individual's week 0 and week 8 sample on the same microarray. We performed 40 Agilent microarrays for 20 GH treated men and women. Validation of the Agilent microarray expression data was performed for seven genes using quantitative real time PCR (qPCR). For both gene expression profiling platforms, the GH-induced alterations were small, and similar in magnitude to variations between individuals. Results from both platforms demonstrate that maximal regulation was of the order of two fold, and that few genes were regulated greater than 1.5 fold. Further, whilst both platforms identify differentially expressed genes, these signatures were different between men and women treated with GH. Using data from the Affymetrix platform at the probe set level, testosterone alone had little effect, whilst the administration of testosterone and GH together resulted in regulation of probe sets that showed little overlap with those regulated by GH alone.

We conclude that it is unlikely that gene expression analysis of peripheral blood leukocytes would be a viable approach for the detection of GH doping.