PROJECT REVIEW

"The effect of training, altitude exposure and an athlete's sex on expression of genes known to change following autologous blood transfusion"

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Training diaries and documents seized during raids associated with 'Operation Puerto', together with the discovery of blood transfusion equipment at the Turin 2006 Winter Olympics, has revealed systematic use of autologous blood transfusion by elite athletes. No test currently exists to detect this banned practice.

Substantial progress has been made by the SIAB research consortium in projects previously funded by the WADA to identify genes that are switched on or off following blood transfusion. Changes in the expression of these genes may thus serve as a diagnostic test to detect the use of autologous transfusion. Further research is required to demonstrate that candidate genes are not perturbed by non-doping activities, such as altitude exposure which may have a similar physiological consequence to blood transfusion. Consequently, this project will collect blood samples from elite athletes at the Australian Institute of Sport to quantify how much the identified gene markers vary from day-to-day during both typical training regimens and altitude exposure. Samples will be collected from both men and women athletes to study the effect of an athlete's sex on gene markers.

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Results and Conclusion

Autologous blood transfusion is one of the most effective methods of illicit performance enhancement for endurance athletes. For instance the USADA report on Lance Armstrong, released on October 10 2012 (http://cyclinginvestigation.usada.org/), indicates widespread systematic blood doping in the professional cycling peloton for at least a decade. Hitherto, no method has been effective in reliably detecting this banned practice.

This study was based on previous WADA funded research by the Science and Industry Against Blood-doping (SIAB) consortium, which identified via microarray techniques, 16 genes that showed significant alterations in transcript abundance following autologous blood transfusion in healthy volunteers.

The aim of this study was to assess day-to-day variation of identified gene markers in blood samples from elite male and female athletes at the Australian Institute of Sport (AIS), collected during both typical training regimens and altitude exposure, as a basis for evaluating the usefulness and limitations of using gene expression profiles as indirect markers of autologous transfusion. An additional 25 genes, involved in relevant biological pathways, were added to the initial set of candidate markers to provide a candidate set of 41 genes. Common data-mining tools were used to differentiate between the gene-signatures of transfused subjects (from previous SIAB projects) from those of AIS athletes undergoing different training modalities as well as being sampled at different times of the day.

We demonstrated that a subset of 16 of the 41 genes (not the same 16 identified by SIAB) were significantly affected by either the between-subject factors of sex and training mode and/or the within-subject factors of being fasted and time of the day of sampling. Other genes proved to be stably expressed and unaffected by either between- and/or within-subject effects in our experimental setting. We also showed that altitude training affected

expression levels of some of the markers to the same extent as transfusion, whilst others were robust and not affected by altitude exposure. Finally, we determined that within-subject variation was significantly smaller than between-subject variation for a substantial number of genes, indicating that for diagnostic purposes, individual follow-up and, for instance, inclusion of expression markers into an Athlete Biological Passport approach should be preferred over set population cutoff values.

Overall, our findings indicated that transfusion led to changes in peripheral blood transcript abundance that are likely to be universal and distinct enough to lead to successful classification of transfused subjects as such, regardless of sex or training mode. However, there were also some false positives (controls classified as transfused) which suggests that, on their own, the discriminative power of the 16 genes is unlikely to be sufficient in the anti-doping context.

In regard to increasing robustness of respective gene signatures as a diagnostic tool, we suggest assessment of global gene expression via RNAseq rather than measurement of a single, or small selection, of genes.