Project Review

"Comparative gene expression profiling in human buccal epithelium and leukocytes after the abuse of beta-2-agonists and anabolic steroids"

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Doping analysis is always in the misery of ethics, minimal invasive tissue collection and the establishment of sensitive, valid and reliable techniques to detect illegal use of forbidden substances in sports.

Until now GC-MS is the technique of choice to quantify the banned substances or their metabolites. But in the last years, modern techniques of molecular biology are in development to become alternative or supplemental choice to elucidate the abuse of diverse doping substances.

Therefore, the combination of diversified screening of gene expression by high throughput gene array analysis, evaluation of array data by quantitative RT-PCR, information transfer to customized cheap gene array and the verification by established methods such as GC MS would be a useful synergy in the fight against doping.

The following research application includes several subprojects. In appreciation of ethic recommendations of tissue collection, buccal epithelium, lymphocytes and leukocytes could serve as minimal invasive RNA source for the proposed comparative molecular biology analysis. The main focus is located on the evaluation of a fast and reproducible method to isolate sufficient amounts of intact total RNA out of the tissues by RNA-stabilizing chemical extraction techniques. The second step is to screen the two tissues for possible side effects by physical activity. Therefore, baseline results should be generated by a group of control sportsmen. Following, three additional populations, asthmatics, anti aging patients (and/or patients with testicular hypofunction) and bodybuilders, should be tested for either the use of inhaled beta-2-agonists or anabolic steroids by gene array, RT PCR and GC-MS in comparison to the control population.

The main objective of the present study is to evaluate an innovative valid long term screening technique to elucidate candidate genes which are modulated by illegal substances and not by factors such as physical activity or circadian rhythm.

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Results and Conclusions

The current test results demonstrate that gene array analytics has not yet reached the aspired quality level to guarantee a constant reproducibility over time–particularly when involving batch removal. In addition, the low level of reproducibility may lead to the discrepancy between gene array and RT-PCR results. Realtime RT-PCR revealed high reproducibility. Results were subject to intense biological variation in relation to exercise load, time and hormone substitution. Additionally, hormone analyses from the saliva showed a potential method for direct evidence.

Future plans include: Expanded cluster analyses or new mathematical models possibly elicit further target genes that have to be validated via RT-PCR.