VALIDATION OF ALLELIC DISCRIMINATION ASSAYS FOR DRUG METABOLISM

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INTRODUCTION

The goal of this project is to validate a series of genotyping assays in order to investigate drug metabolism genes that may also be associated with trigger points and myofascial pain. We hypothesized that the allelic discrimination assay would be more advantageous for genotyping the candidate genes as an alternative to sequencing, which is currently the gold standard. Genotyping studies were performed using a sample size of 19 subjects. Subject DNA were previously isolated and de-identified prior to assignment. Genotyping was performed using TaqMan allelic discrimination assays (Applied Biosystems) on the StepOne real-time PCR instrument. To confirm the genotype determined, a percentage of the samples were analyzed by Sanger sequencing. The GenoTyper app was used for comparison to StepOne software for genotyping. We were able to obtain 100% accuracy when comparing the allelic discrimination assay to the sequencing method. 100% reliability was obtained within assay for both SNPs with 100% reliability for the CYP2C9 SNP and 88% reliability for the CYP1A2 SNP between assays.

METHODS

Genotyping studies were performed with a sample size of 19 subjects. Subject DNA were previously isolated and de-identified prior to assignment. Genotyping was performed using TaqMan allelic discrimination assays (Applied Biosystems) on the StepOne real-time PCR instrument. To confirm the genotype determined by the TaqMan assay, a percentage of the samples were analyzed by Sanger sequencing. The GenoTyper app was used for comparison to StepOne software for genotyping.

RESULTS

In the rs762551 locus (CYP1A2), the presence of the AA genotype is indicative of a fast caffeine metabolizer. Genotypes AC are carriers, and genotypes CC are slow metabolizers. In the rs1057910 locus (CYP2C9), the presence of the AA genotype indicates a normal metabolizer of warfarin. Genotypes AC are carriers with a 40% reduction in metabolism while CC genotypes are indicative of a poor metabolizer.

In conclusion, we have determined that the allelic discrimination assay validation had not been fully successful due to the software's inability to accurately call genotypes. We believe the reporter dye assignments should be verified with the manufacturer.

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REFERENCES

discussion/pcr-assays/snp-sequencing/4th-street/taqman-assays.html

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