VALIDATION OF ALLELIC DISCRIMINATION ASSAYS FOR DRUG METABOLISM

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ABSTRACT

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. We established our LOD at 10ng/µL of DNA. We could not confidently validate the assay due to the software's inability to automatically call the genotype without manual assistance from the use of the multi-component plot. Hence, we were led to seek an alternative software to predict the genotype without manual interference. 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. For future studies, we recommend the use of the GenoTyper (GT) app for accurately identifying the genotype due to its advanced algorithm. Further validation is necessary for this new algorithm before adopting this method.

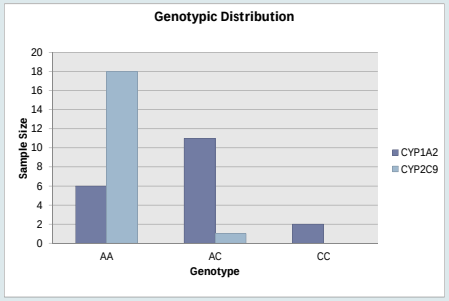
INTRODUCTION

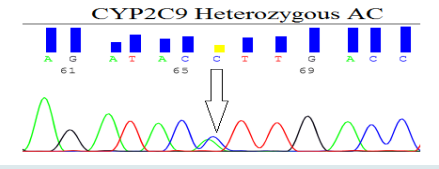
Myofascial trigger points (TrPs) are described as very tender spots in taut bands of hardened muscle. A TrP may result in myofascial pain syndrome, which is a combination of sensory, motor, and autonomic symptoms. The biochemical cascade that occurs in the formation of TrPs is complex, resulting in a disruption of normal function in the neuromuscular junction. By understanding the interactions of these molecular players in an active TrP, we can better prevent TrP development and also inactivate and eliminate existing TrPs.

We are investigating two genes associated with drug metabolism that may also be associated with trigger points and myofascial pain: CYP1A2 (rs762551) and CYP2C9 (rs1057910). The goal of this project is to validate a series of genotyping assays for candidate genes for susceptibility to muscle trigger point development, using preordered assays and designing Sanger sequencing assays for each gene to confirm the results of the allelic discrimination assay.

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.

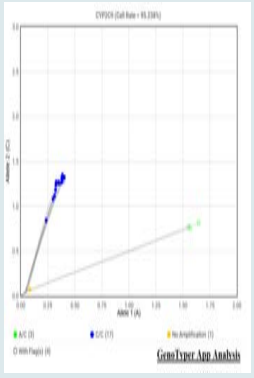
After genotyping using the allelic discrimination assay, followed by Sanger confirmation, we were able to obtain 100% concordance with the CYP2C9 SNP and 100% concordance with the CYP1A2 SNP. After Sanger confirmation, we discovered that the reporter dye assignments were reversed in comparison to what was stated on the assay data information sheet provided by the manufacturer. However, we can not confidently validate the assay due to the software's inability to automatically call the genotype without manual assistance from the use of the multi-component plot. Hence, we were lead to seek an alternative software to predict the genotype without manual interference.

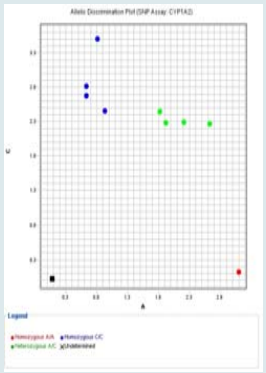
CYP1A2 (rs762551) Genotype Statistical Analysis

Accuracy	Precision		Blind Sample	Sanger Confirmation
	Within	Between		
100%	100%	88%	80%	100%

CYP2C9 (rs1057910) Genotype Statistical Analysis

Accuracy	Precision		Blind Sample	Sanger Confirmation
	Within	Between		
100%	100%	100%	100%	100%





CONCLUSION

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.

Some of the issues faced during the validation of this assay were limited reagents, contamination, and sample quality. These issues hindered the proficiency efforts of our results and should be addressed in future validation studies.

For future studies, we recommend the use of the GenoTyper (GT) app for accurately identifying the genotype due to its advanced algorithm. Further validation is necessary for this new algorithm before adopting this method.

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