NAT2 ALLELE FREQUENCY IN AMERICAN INDIAN POPULATION

KELSEY CABLE, MARK PERSHOUSE
Center for Environmental Health Sciences,
The University of Montana, Missoula, MT 5980145

Abstract

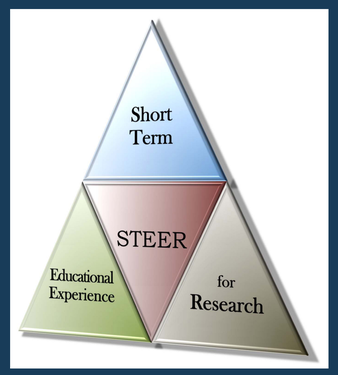
The NAT2 gene functions to both activate and deactivate arylamine and hydrazine drugs and carcinogens. Thus, NAT2 activity is associated with drug effects, toxicities and susceptibility to various cancers. Polymorphism in the NAT2 gene result in rapid or slow acetylator phenotypes. 36 different alleles of the NAT2 gene have been identified so far, but not all of these sequence variations lead to changes in the enzyme activity of the encoded protein. The purpose of this study was to characterize interethnic variability in the frequency of the NAT2 allele in the Confederated Salish and Kootenai (CSK) population. Fifty-five blood samples were assessed for their genotype by isolating the DNA from peripheral blood, performing PCR, digestion with restriction enzymes, and then analyzing the DNA fragments on the 2100 bioanalyzer, a capillary electrophoresis unit for low-throughput fragment sizing. The analysis showed differences in the allele frequencies of the CSK tribe when compared to Caucasian population.

Introduction

Pharmacogenomics examines inherited variation in genes that dictate drug metabolism and response. According to a study published in the Journal of American Medical Association, adverse drug reactions (ADRs) account for more than 2.2 million serious cases and over 100,000 deaths in the United States each year. Many of these ADRs result from single nucleotide polymorphisms (SNPs) that change enzyme activity. SNPs in the genes encoding enzymes dictate the efficiency of how the enzyme processes toxicants and drugs. Decreased metabolism can lead to toxicity and the build up of carcinogens. Increased metabolism may lead to ineffective drugs.

N-acetyltransferace is a phase II metabolizing enzyme, which is expressed at high levels in the intestine and liver. NAT2 participates in the detoxification of many hydrazine and arylamine drugs. NAT2 catalyzes the N or O-acetylation of various arylamine and heterocyclic amine substrates and is able to bioactivate several known carcinogens.

While the NAT2 variant allele frequencies has been characterized in many ethnic populations, it has not been characterized in the American Indian Populations. Our specific focus because of longstanding collaborations is on the Confederated, Salish & Kootenai tribes (CSKT). These variant allele frequencies are needed for prescribing the most effective aryl amine and hydrazine drugs, while lowering the risk of ADRs. In addition, these frequencies play a vital role in understanding a possible increased risk of cancer from carcinogens in cigarette smoke. The aim of this study is to characterize the variant allele frequency for CSK tribal members. This characterization will provide tribal members with the possible of an equal of healthcare, already available to other ethnicities.



Acknowledgements

The project described was supported by Award Number R25ES016247 from the National Institute Of Environmental Health Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Environmental Health Sciences or the National Institutes of Health.

Experimental approaches and methods

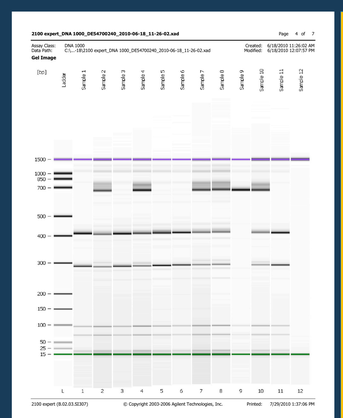
Isolated DNA blood samples from CSK tribal members were tested. The polymorphic alleles of the human NAT2 promoter samples were amplified from the DNA samples using polymerase chain reaction (PCR) as in Deitz et al. 2.0ul of genomic DNA were used as the template.

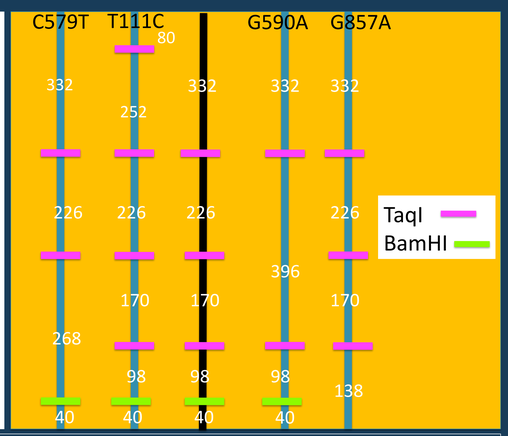
The PCR products were digested with various enzymes .

Digested PCR products were run on the 2100 bio-analyzer.

The length of the uncut PCR product was 866 bp. After digestion different bp strands resulted.

TaqI and BamHI digest





Left Picture: Electropherogram of sample set 1 after digestion with enzymes TaqI and BamHI.
Right Picture: Map of the 3 polymorphisms shown after digestion with TaqI and BamHI.

NAT2 Gene **Sequence**

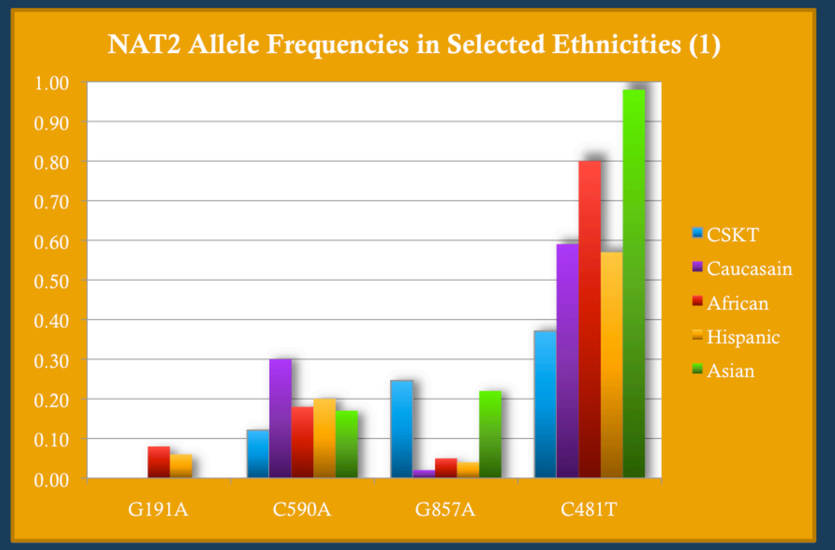
atggacattg aagcatattt tgaaagaatt ggctataaga actctaggaa caaattggac ttggaaacat taactgacat tcttgagcac cagatc\cgg g ctgttccctt\ tgagaacctt aacatgcatt gtgggcaagc catggagttg ggcttagagg ctattt\tga tcacattgta agaagaaa c\c gg ggtgggtg gtgtctccag gtcaatcaac ttctgtactg ggctctgacc acaatcggtt ttcagaccac aatgttagga gggtattttt acatccctcc agttaacaaa tacagcactg gcatggttca ccttctcctg caggtgacca t\tgacggcag gaattacatt gt\cgatgctg ggtctggaag ctcctcccag atgtggcagc ctctagaatt aatttctggg aaggatcagc ct\ agg tc\c ttgcattttc tgcttgacag aagagagagg aatctggtac/ c tggaccaaa tcaggagaga gcagtatatt acaaacaaag aatttcttaa ttctcatctc ctgccaaaga agaaacacca aaaaatatac ttatttacgc ttgaacct\cg a acaattgaa gattttgagt ctatgaatac atacctgcag acgtctccaa catcttcatt tataaccaca tcattttgtt ccttgcagac cccagaaggg gtttactgtt tggtgggctt catcctcacc atagaaaat tcaattataa agacaataca gatctgg t\cg a gtttaaaac tctcactgag gaagaggttg aagaagtgct gagaaatata tttaagattt ccttggggag aaatctcgtg cccaaacctg gtgatg\gatcc cttactatt taga ataagg aacaaaataa acccttg tgt atgtatcacc caactcacta attatcaact tatgtgctat cagatatcct ctctaccctc

866bp NAT2 gene sequence showing enzyme cut sites as well a forward and reverse primers. Possible cut sites are white with a backslash corresponding to the color of the enzyme.
MspI
KpnI
TaqI
BamHI
AciI

Original Primers

Nested Primers

Results



Population	Variant Frequency						
	G191A	A434C	C590A	G857A	T111C	C481T	C759T
CSKT	0%	0%	12.1%	24.6%	0%	37.1%	0%
Caucasian	0%	ND	30%	2%	ND	59%	ND
African	8%	ND	18%	5%	ND	80%	ND
Hispanic	6%	ND	20%	4%	ND	57%	ND
Asian	0%	ND	17%	22%	ND	98%	ND

Conclusion

- Compared to four other ethnic groups, the frequency of NAT2 variant alleles is unique in the CSK tribal population.
- Based on these results, response to arylamine and hydrazine drugs and the possibility of ADRs would not be similar to other ethnic groups, so testing and verification of genotype/phenotype correlations is warranted.

Future Directions

- Analyze data to determine if these results are statistically significant.
- Find the remaining variant alleles in these 55 samples, to fully characterize the predicted NAT2 enzyme as a slow or fast metabolizer.
- Confirm these results, by genotyping other CSKT blood samples.
- Determine frequency of variant alleles in related genes involved in the metabolism of carcinogens from cigarette smoke.
- Determine frequency of variant alleles in other tribal populations.

References

1). dbSNP database: http://www.ncbi.nlm.nih.gov/projects/SNP/
http://www.thesgc.org/structures/structure_description/2PFR/