Analysis of the CYP1A2 Caffeine Metabolism Gene in the LSSU Student Population

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Abstract

Eighty-five percent of Americans drink caffeinated beverages on a daily basis. Each individual responds differently to caffeine depending on age, gender, diet, and ethnicity. Caffeinated beverages cause insomnia in some people, but not in others due to differences in the rate of caffeine metabolism. This study set out to examine variation in the caffeine metabolism of Lake Superior State University (LSSU) students. My hypothesis was that LSSU student allele frequencies would match those of the general population: 47.5% fast, 41.0% medium, and 11.5% slow caffeine metabolism. I sampled 200 LSSU students via buccal swabs. DNA was successfully isolated from 164 samples. Participants filled out a demographic questionnaire entailing caffeine intake, ethnicity, and sex. The CYP1A2 gene was amplified via standard PCR prior to genotyping by restriction digest and gel electrophoresis. The APAI restriction enzyme was used to determine the genotype of the rs762551 single nucleotide polymorphism (SNP), while the SACI enzyme was used as a positive digestion control. Overall, results showed a total of 42.7% fast, 44.5% medium, and 12.8% slow metabolizers. Of special note is that 24 of the 164 students sampled were of Native American heritage, an important yet underrepresented group in human genomics. This study provides the first reported look at the CYP1A2 variation within this North American subpopulation with metabolism rates being 50% fast, 33.3% medium, and 16.7% slow. The results confirm my initial hypothesis that the variation of caffeine metabolism gene frequencies for the LSSU student population would be representative of published allele frequencies for the general US population.

Background

Caffeine is a white powdery substance with a chemical structure of 1,3,7-trimethylxanthine (IMCMNR, 2001). Caffeine itself was first isolated in 1819 by Friedlieb Ferdinand Runge and is now recognized as the most used psychoactive drug worldwide (Weinberg et al., 2001). In the United States alone, 85% of individuals consume at least one caffeinated drink every day (Mitchell et al., 2014). Each individual will respond differently to caffeine depending on a variety of factors including age, gender, diet, ethnicity, and Body Mass Index (Nehlig, 2018). Some people can consume a caffeinated beverage before bed and still fall asleep, while others will be awake for hours. This difference in response is determined by the body's ability to metabolize caffeine. The gene that encodes for caffeine metabolism is found on the CYP1A2 gene, which can be found on Chromosome 15, located at the loci 15q24 (Cornelis et al., 2011). This hepatic cytochrome enzyme is responsible for the metabolism of a number of substrates that are important in the human body. Substrates like procarcinogens, hormones, drugs, endogenous compounds (Chernyak et al., 2011). The base pair substitution from A to C in the CYP1A2 gene changes the metabolism rate. The homozygous allele (AA) metabolizes caffeine at a fast rate, the heterozygous allele (AC) metabolizes caffeine at a medium rate, and the homozygous allele (CC) metabolizes caffeine at a slow rate (Zephyr and Walsh, 2015). The single nucleotide polymorphism (SNP) determines differing levels of enzyme function. This particular SNP (rs762551) is in an intron of DNA that can increase transcription rates and increase mRNA translation efficiency (Shaul, 2017). Restriction fragment length polymorphism (RFLP) is a type of polymorphism that results from DNA sequence variation and is recognized by the restriction enzyme. This results in three separate fragment lengths with base pairs at 249, 494, and 743.

Objectives

The objective of this study is to examine variation in the caffeine metabolism of LSSU students. My hypothesis that that the LSSU student allele frequencies would match those of the general population: 47.5% fast, 41.0% medium, and 11.5% slow caffeine metabolizers.

Methods

- 200 DNA samples were collected from students via buccal swab at LSSU in Sault Ste. Marie, Michigan. DNA was successfully extracted from 164 of the samples.
- All participation was on a voluntary basis. Each subject received and signed a copy of the consent form approved through the Institutional Review Board for the Protection of Human Subjects at LSSU. Students also filled out an anonymous demographic questionnaire detailing questions regarding age, gender, ethnicity, and caffeine consumption. Students were all at least 18 years of age and active full-time or part-time LSSU students.
- Genomic DNA was isolated using the Monarch Genomic DNA Purification Kit (NEB) Sample Lysis: Animal Tissue protocol.
- Polymerase Chain Reaction (PCR) amplification was performed using published primers for the CYP1A2 gene.

Methods

- The restriction enzymes used to digest the Post-PCR product were APAI and SACI. The APAI enzyme was used detected the SNP and cut the allele only if the C base pair was present. It did not digest alleles containing A. This resulted in three bands present in fragment analysis with base pairs at 249, 494, and 743 (Zephyr and Walsh, 2015). The SACI enzyme served as the control restriction digest with base pairs appearing in fragment analysis at 249 and 494.
- Fragment analysis was completed using standard gel electrophoresis.

Results

Table 1: Metabolism rates shown below according to population and subpopulation.

- *All values less then the critical Chi Square: 3.841.*
- *Not significantly different from published allele frequencies.*

Population	Actual			Experimental
	Fast	Medium	Slow	χ2 Value
European	43.80%	43.20%	12.90%	0.3914
African American	25%	50%	25%	0.2447
Asian	12.30%	57.10%	28.60%	3.786
Native American	50%	33.3%	16.7%	0.923
Finnish	54.50%	33.30%	18.20%	0.9642
British	53.60%	39.30%	7.10%	0.1651

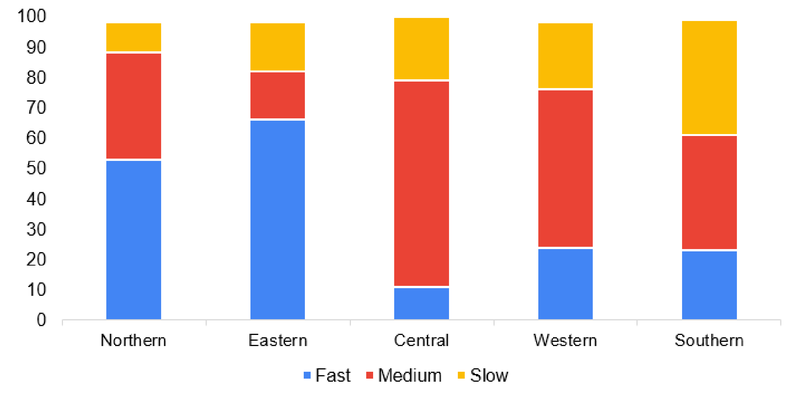

Figure 2: Breakdown of Europe into geographic regions.

- *This shows that caffeine metabolism varies across geographic distribution.*
- *Northern & Eastern Europe have more fast metabolizers*
- *Central & Western Europe have more medium metabolizers*
- *Southern Europe has equal distribution of medium and slow metabolizers*

Discussion

The results confirm my initial hypothesis that the variation of caffeine metabolism gene frequencies for the LSSU student population would be representative of published allele frequencies for the general US population. The European, African American, Asian, Finnish, and British population experimental Chi-Square values were less than the critical Chi Square value of 3.841. Thus, the major population and the subpopulations at LSSU are not significantly different from published allele frequencies. Age and sex also had no significant differences from the European allele frequencies.

Discussion

Population	Fast	Medium	Slow
Predicted	47.5%	41.0%	11.5%
Overall	42.7%	44.5%	12.8%
Native American	50%	33.3%	16.7%

Of special note is that 24 of the 164 students sampled were of Native American heritage, an important yet underrepresented group in human genomics. This study provides the first reported look at the CYP1A2 variation within this North American subpopulation with metabolism rates being 50% fast, 33.3% medium, and 16.7% slow.

Knowing your caffeine metabolism could improve your health. Consuming too much caffeine can cause negative side effects such as: increased blood pressure, insomnia, heart palpitations, dehydration, headaches, nervousness, irritability, and muscle tremors. Slow metabolizers should avoid excessive caffeine consumption as they are at risk for heart attacks. There is the possibility of interactions with medications and supplements. Such medications interactions include Quinolones which decrease caffeine metabolism and Bronchodilators because both are stimulants.

Acknowledgments

I would like to thank to these individuals for their invaluable help that made this project possible:

- Stephen H. Kolomyjec, PhD, LSSU, for the guidance, advice, and insight as my project mentor.
- Britton D. Ranson Olson, PhD, LSSU Advisor
- Martha A. Hutchens, PhD, LSSU Advisor
- Haven Borghi, Hannah Sawicki, and Jacob Pittsley for their help collecting samples and conducting research.

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