Differences in Allele Frequency Between Hmong and East Asian Population for Key Genetic Variants within Very Important Pharmacogenes

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Introduction

- Implementing pharmacogenomics (PGx) for **very important pharmacogenes (VIPs)** can optimize medication selection and thus improve clinical outcomes.
- The National Institutes of Health *"All of Us"* initiative seeks to include under-served and under-studied populations.
- Hmong people represent an under-studied ethnically distinct sub-population of Southeast Asians residing in the US who exhibit specific health disparities.[1-5]
- Failure to recognize differences in allele frequencies between sub-populations may lead to erroneous decisions regarding drug and dose selection.
- **Research Aim:** To determine allele frequencies of key single nucleotide polymorphisms (SNPs) within high priority VIPs and compare those frequencies in the Hmong with an East Asian population.
- **Significance**: This is the first comprehensive PGx testing conducted in Hmong.

Methods

- **Study Design**: Quantitative cohort study of Hmong residing in Minnesota and Wisconsin.
- Saliva from 198 self-identified Hmong (**Table 1** and **2**) was collected using ORAgene® saliva kits and analyzed for 22 SNPs on 8 genes (*CYP2C9*, *CYP2C19*, *CYP4F2*, *DYPD*, *G6PD*, *SLCO1B1*, *TMPT*, *VKORC1*) by TaqMan® implemented on a Biomark HD platform (Fluidigm Inc.) within a CLIA-certified laboratory.
- Chi-Square or Fisher's exact test (corrected *p*-value 0.002) was used for frequency comparisons for the Clinical Pharmacogenetics Implementation Consortium (CPIC) actionable variants within VIPs between our Hmong participants and an East Asian population from CPIC database or Genome Aggregation Database (gnomAD).
- Study was approved by University of Minnesota IRB (#1702M06041).

Table 1. Characteristics of participants

	N = 198
Age, years (mean ± S.E., range) *(n=196)*	32.6 ± 1.36 (18-94)
Gender, male N (%)	77 (38.9)
Height, inches (mean ± S.E., range) *(n=197)*	62.1 ± 0.25 (53.8-70.0)
Weight, lb (mean ± S.E., range) *(n=197)*	148.5 ± 2.3 (82-288)
BMI, kg/m² (mean ± S.E., range) *(n=197)*	27.1 ± 0.38 (16.3-53.8)
Birth country	
USA, N (%)	115 (58.4)
Laos, N (%)	50 (25.4)
Thailand, N (%)	32 (16.2)
Preferred language	
English, N (%)	124 (62.6)
Hmong, N (%)	28 (14.1)
Number of years in the U.S., years (mean ± S.E., range) *(n=82)*	27.3 ± 1.16 (1-45)
Individual taking at least one medications/supplements, N (%)	**89 (44.9)**
Medication = 1, N (%)	50 (56.2)
Medication = 2, N (%)	11 (12.3)
Medication > 3, N (%)	7 (7.9)

Table 2. Medications Taking History

	Count
Number of prescription medications	**77**
Number of clinical actionable medications determine by CPIC (omeprazole, sertraline, simvastatin, tacrolimus, warfarin, and other antidepressants)	**10**
Number of over-the-counter	**66**
Number of unknown medications	**26**

Table 3. Allele Frequencies Comparison within VIPs

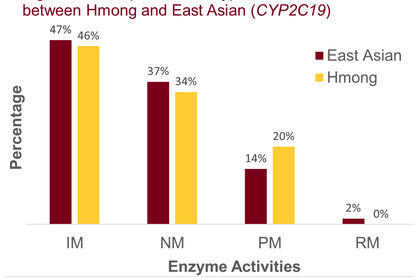
Genes	Common Allele[a]	dbSNP Number[a]	Nucleotide Change[a]	Protein Effect[a]	Enzyme Activity[a]	Frequency, %		*p*-value
						Hmong	East Asian[b]	
CYP2C9	*1	-	Wild-type	None	Normal	83.4	96.6	**<0.001[d]**
	*2A	rs1799853	c. 3608C>T	R144C	Decreased	0	0.06	1
	*3A[c]	rs1057910	c. 42614A>C	I359L	Decreased	16.6	3.8	**<0.001[d]**
CYP2C19	*1	-	Wild-type	None	Normal	57.2	56.1	**0.81[d]**
	*2	rs4244285	c. 19154G>A	Splicing defect	None	42.2	29.0	**<0.001[d]**
	*3	rs4986893	c. 17948G>A	W212X	None	0.3	9.3	**<0.001**
	*4A/*4B	rs28399504	c. 1A>G	M1V	None	0	0.3	1
	*5	rs56337013	c. 90033C>T	R433W	None	0	0.4	1
	*6	rs72552267	c. 12748G>A	R132Q	None	0.3	0.02	0.27
	*8	rs41291556	c. 12711T>C	W120R	None	0	0	1
	*17	rs12248560	c. -806C>T	Increased expression	Increased	0	1.3	0.14
CYP4F2	*1	-	Wild-type	None	Normal	93.5	80.0[e]	**<0.001[d]**
	*3	rs2108622	c. 1297G>A	V433M	Decreased	6.5	22.1	**<0.001[d]**
DPYD	*1	rs67376798	Wild-type	None	Normal	100	98.2[e]	0.053
	*2A/*2B	rs3918290	c. 1905+1G>A	N/A	None	0	0	1
	*13	rs55886062	c. 1679T>G	I560S	None	0	0	1
G6PD	B	-	Wild-type	None	Normal	100	95.3[e]	-
	-	rs1050828	c. 202G>A	V68M	Deficient	0	0	1
	-	rs5030868	c. 563C>T	S188F	Deficient	0	0	1
	-	rs72554665	c. 1376G>T/C	R459L/P	Deficient	0	0.2	0.055
SLCO1B1	*1a/*1b	-	Wild-type	None	Normal	96.4	85.9	**<0.001[d]**
	*5[c]	*rs4149056*	c. 521T>C	V174A	Decreased	3.6	0.1	**<0.001[d]**
TPMT	*1	-	Wild-type	None	Normal	99.4	98.4	0.38
	*2	rs1800462	c. 238G>C	A80P	None	0	0	1
	*3B	rs1800460	c. 460G>A	A154T	None	0	0	1
	*3C	rs1142345	c. 719A>G	Y240C	None	0.6	1.6	0.25
	*4	rs1800584	c. 626-1G>A	None	None	0	0	1
VKORC1	-	rs9923231	c. -1639G>A	-	Decreased	88.1	88.2	0.33[d]

[a] Data from CPIC database or PharmVar; [b] East Asian data was obtained from CPIC resource or gnomAD; [c] Variant alleles significantly deviate from Hardy-Weinberg equilibrium (*p*-value for *CYP2C9**3: 0.004; *SLCO1B1**5: 0.023); [d] Using Chi-Square test with Yates' continuity correction; otherwise, Fisher exact test was used; [e] Estimated frequency; Bold and gold background: Bonferroni-corrected $p \leq 0.002$

Table 4. CPIC Supported Drug-Gene Pairs

Genes	Drugs
CYP2C9	Warfarin (with *VKORC1*, *CYP4F2*) Phenytoin (with *HLA-B*)
CYP2C19	Citalopram, Clopidogrel, Sertraline, Voriconazole; With *CYP2D6*: Amitriptyline, Clomipramine, Doxepin, Imipramine
DPYD	Capecitabine, Fluorouracil, Tegafur
G6PD	Rasburicase
SLCO1B1	Simvastatin
TPMT	Azathioprine, Mercaptopurine, Thioguanine

Figure 1. Example of Phenotype Differences between Hmong and East Asian (*CYP2C19*)



IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; RM, rapid metabolizer

Results

- Significant differences ($p < 0.002$) in allele frequencies between the Hmong and East Asians were noted in 29% (8/28) of the CPIC actionable variants tested. (**Table 3**)
- All study subjects carry at least one CPIC actionable variant.

Conclusion

- Statistically significant differences in allele frequencies for key SNPs influencing medication dosage and selection exist between the Hmong and East Asians
- These differences may translate into different medication recommendations for the Hmong, relative to other East Asian populations.
- **Interpretation**: These differences underscore the importance of individualizing genetic information and including all communities in PGx research.

Discussion and Future Directions

- The drug-gene pairs selected in this study was based on CPIC guidelines which aims to assist clinicians in understanding how available genetic test results should be used to optimize drug therapy.
- We intend to expand the list of VIPs in a future study (e.g. CYP2D6, CYP3A5, HLA, CFTR, and UGT1A1).
- Sequencing key pharmacogenes in the Hmong may identify novel variants worthy of further investigation.
- To advance the science of pharmacogenomics within under-studied or under-served populations, investigators should employ culturally and linguistically sensitive approaches.
- **Limitations**: Two SNPs (rs1057910 and rs4149056) were not in Hardy-Weinberg equilibrium despite using two independent genotyping methods (TaqMan and Sequenom) to verify the genotyping results.
- We cannot exclude the possibly of relatedness among participants due to the community structure (clans).

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