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Assessment of genetic polymorphisms associated with hyperuricemia or gout in the Hmong

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Abstract

Aim—Hyperuricemia commonly causes gout. Minnesota Hmong exhibit a two- to fivefold higher prevalence of gout versus non-Hmong. To elucidate a possible genomic contribution to this disparity, prevalence of risk alleles for hyperuricemia in Hmong was compared with European (CEU) and Han-Chinese (CHB).

Methods—In total, 235 Hmong were genotyped for eight SNPs representing five candidate genes (*SLC22A12*, *SLC2A9*, *ABCG2*, *SLC17A1* and *PDZK1*).

Results—The frequency of seven out of eight risk alleles in the Hmong was significantly different than CEU; six higher and one with lower prevalence. The frequency of three out of eight risk alleles in the Hmong was significantly different than CHB; two higher and one with lower prevalence.

Conclusion—Hyperuricemia risk alleles are more prevalent in the Hmong than CEU and HB.

Keywords

Hmong; hyperuricemia; SNPs

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Disclosure

Some of this work was presented as an abstract at the Arteriosclerosis, Thrombosis, and Vascular Biology (ATVB) Conference in May 2014 in Toronto, Canada.

Competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Background

The global prevalence of hyperuricemia has grown dramatically over the past two decades [1]. In the USA, the prevalence of hyperuricemia significantly increased from 18.2% in 1988–1994 to 21.4% in 2007–2008 [1–3]. Hyperuricemia is typically classified by serum uric acid (SUA) ≥ 6.0 mg/dl and is the strongest predictor of gout, the most common inflammatory arthropathy disease [4–6]. When SUA level exceeds its solubility threshold (> 6.8 mg/dl), the risk of monosodium urate crystal formation is high and often leads to a variety of acute and chronic complications. Although fundamentally a metabolic disorder, hyperuricemia has also been strongly associated with increased risk for cardiovascular diseases, chronic kidney disease, renal stones and new onset of Type 2 diabetes mellitus, which all have a significant impact on morbidity and mortality [7–9].

Hyperuricemia results from an imbalance between the production and excretion of SUA. Overproduction of uric acid (UA) may be attributed to diets rich in purine content [10] or pathological conditions leading to accelerated breakdown or production of endogenous nucleic acids. While breakdown of endogenous purines represents the main sources of SUA, underexcretion of UA is typically the most common cause of hyperuricemia [11]. UA is primarily excreted renally with only 30% being eliminated nonrenally. Specifically, UA excretion takes place in the proximal convoluted tubule, yet the majority (~90%) of filtered UA is reabsorbed in the descending renal tubule [11]. UA excretion is confounded by declining kidney function, concomitant use of select drugs (e.g., niacin, low-dose aspirin, thiazides and loop diuretics) and urine pH. Several nonmodifiable factors such as age, gender and race can also influence SUA levels.

Considerable variability in the prevalence of hyperuricemia and gout across various races and ethnicities has been described [12]. For example, African-Americans [13], Maori [14], Japanese [15] and Hmong [16] have each been shown to have a higher prevalence of hyperuricemia or gout relative to Caucasians, raising the suspicion that there is a genetic basis for this observation [14]. These observations are supported by studies that have identified a differential prevalence of SNPs within genes affecting the urate transportome [13,17,18], which is in part responsible for elevated SUA levels [19–23]. Genome-wide association studies (GWAS) have identified transporters including *SLC22A12*, *SLC2A9*, *ABCG2* and *SLC17A1* as well as the scaffolding protein *PDZK1*, to have the highest degree of impact on SUA [24]. Although other genes are involved, their mechanisms of contribution to elevated SUA remain unclear [25,26].

Minnesota Hmong represent an important, underserved minority group numbering over 64,000 [27]. Hmong patients suffer from a twofold higher prevalence of gout [28] and fivefold higher incidence of UA ureteral and kidney stones [16,29], compared with non-Hmong Minnesotan patients. In an attempt to ascertain if genetic polymorphisms may contribute to the Hmong's predisposition to hyperuricemia and gout, the frequencies of validated risk alleles within five candidate genes (*SLC22A12*, *SLC2A9*, *ABCG2*, *SLC17A1* and *PDZK1*) were quantified in a Hmong cohort, and compared with published data from a racially concordant population of Han-Chinese in Beijing (CHB) and a racially discordant population of Utah residents with ancestry from northern and western Europe (CEU). In

addition, the association between baseline SUA concentration and risk alleles was explored within a subset of the Hmong cohort.

Methods

Study participants

Using the principles of community-based participatory research [30], we created and partnered with the Hmong Genomics Board, which codesigned the study, recruited the participants and collected the data. From March 2009 through May 2009, self-reported Hmong adults 18 years of age were recruited in various settings including; medical clinics, three college campuses, a Minnesota Hmong college student conference and a Hmong national conference held in Wisconsin (IL, USA). As part of the consent process, people received written and verbal information in Hmong or English about what genes are, how they vary in populations and why they might affect health and medication use. Once informed consent was obtained, saliva samples were collected using ORAgene® DISCOVER kits (OGR-500, DNA Genotek, ON, Canada), as per manufacturer's protocol. In addition, demographic information (age, gender, years in USA, formal education), medical history (past medical history, medicines and family history) and anthropometric measurements (heights, weights, waist circumference and blood pressure) were collected. Additionally, although not the primary purpose of this study, we offered to perform blood tests (specifically SUA) on participants 30 years of age and based on their willingness to provide blood samples. This project was approved by the University of Minnesota Human Research Protection Program (UMN IRB # 0711M21884; MN, USA).

SNP selection criteria

Three primary GWAS papers investigating the association of SNPs with SUA were used to guide our approach to selecting the primary genes and relevant SNPs. The first was a GWAS by Vitari *et al.* [31], which included 986 Croatian's isolate and identified *SLC2A9* to be associated with SUA levels, fractional excretion of UA (FEUA) and gout. The second was a meta-analysis by Kolz *et al.* [17], which included 28,141 subjects of European (CEU) descent and identified nine genes and nine loci to be associated with SUA levels. The third study was another meta-analysis by Kottgen *et al.* [18], consisting of over 140,000 subjects from a CEU ancestry. Kottgen *et al.* [18] identified 18 new loci in association with SUA using Gene Relationships Across Implicated Loci approach. The latter meta-analysis is significant for two reasons. First, it provided validation of previously identified SNPs associated with elevated SUA and augmented that data with another phenotype – namely FEUA. Furthermore, it specifically evaluated whether the observed SNP associations were generalizable to individuals of non-CEU ancestry. By replicating their findings within large cohorts of African-Americans, Indians and Japanese populations, it concluded that the SNP effects on SUA concentrations were comparable in magnitude and identical in direction for the majority of SNPs studied [18]. Therefore, key SNPs identified by any of these studies were considered important to pursue in addition to other SNPs identified by candidate gene approaches using phenotypes such as FEUA, fructose load test and simply the presence of gout [32,33].

The final SNP list for the present study was further filtered by considering only those SNPs in genes directly associated with UA homeostasis with a reported minor allele frequencies in excess of 5% based on data from CEU [34] and not otherwise in linkage disequilibrium (r^2 0.8) with other SNPs. Our final list included eight SNPs in five genes directly associated with SUA homeostasis.

Genotyping

DNA was purified and extracted from 235 saliva samples. Briefly, 4 ml of saliva was incubated in a water bath at 50°C overnight. Following incubation, the appropriate amount of purifier was added followed by 10 min of ice incubation. The samples were centrifuged for 20 min at maximum speed (4000 rpm) at room temperature. In order to precipitate the DNA, the yielded supernatant after centrifugation was transferred to new centrifuge tubes followed by adding an equivalent amount of ethanol and then kept in a refrigerator overnight. Samples were then centrifuged for 20 min at maximum speed (4000 rpm) at room temperature. The yielded supernatant was then discarded and the DNA pellet was dried for 30 min followed by adding 1 ml of Tris-EDTA buffer placed on a rotator (34 rpm) overnight. Finally, samples were prepared, labeled and analyzed for quality and quantity using an eight-multichannel Nano-drop (ND8000 V.2., Thermo Fisher Scientific, DE, USA). Eight candidate SNPs in five key genes were genotyped at the Biomedical Genomic Center at the University of Minnesota (MN, USA). Six SNPs were genotyped by Sequenom iPLEX Gold method using Bruker Autoflex II MALDI/TOF mass spectrometer (Bruker, MA, USA; rs505802, rs2231142, rs12129861, rs11942223, rs1014290, rs1183201). The other two SNPs (rs3733591, rs734553) were genotyped using TaqMan® (Applied Biosystems™ 7500 Real-Time PCR, CA, USA).

Sequenom genotyping

Genotyping was performed using the iPLEX gold method. We used iPLEX reagents, protocols for multiplex PCR, single-base primer extension (SBE) and generated mass spectra as per the manufacturer's instructions. Multiplexed PCR was performed in 5 µl reactions on 96-well plates containing 10 ng of genomic DNA. Reactions contain 0.5 U HotStar Taq polymerase (Qiagen, CA, USA), 100 nM primers, 1.25X HotStar Taq buffer, 1.625 mM MgCl₂ and 500 µM dNTPs. Following enzyme activation at 94°C for 15 min, DNA was amplified with 45 cycles of 94°C × 20 s, 56°C × 30 s, 72°C × 1 min, followed by a 3-min extension at 72°C. Unincorporated dNTPs were removed using shrimp alkaline phosphatase (0.3 U, Sequenom, CA, USA). SBE was carried out by addition of SBE primers at concentrations from 0.625 (low molecular weight [MW] primers) to 1.25 µM (high MW primers) using iPLEX enzyme and buffers (Sequenom) in 9-µl reactions. Reactions were desalted and SBE products measured using the MassARRAY system, and mass spectra analyzed using TYPER software (Sequenom), in order to generate genotype calls and allele frequencies.

Statistical analysis

Data from the HapMap [34] served as our source for the frequencies of the variant alleles in CEU and CHB populations. The allele frequencies were then compared with Hmong allele frequencies using Pearson's Chi-Square or Fisher's exact test when appropriate based on cell

count. A Bonferroni adjustment was used to account for multiple ($n = 8$) comparisons between risk alleles using a critical value $p < 0.0063$ for significance. In our analysis, the 'risk allele' was defined as the allele that was either associated with higher SUA or could adversely increase SUA levels. For the purpose of this investigation, we defined hyperuricemia as $\text{SUA} > 6 \text{ mg/dl}$. This definition was selected based on the American College of Rheumatology's recommendation to target $\text{SUA} < 6 \text{ mg/dl}$ in patients treated for gout. Ascertaining the association of the risk of hyperuricemia using the SUA cutoff with genotype was calculated using Pearson's Chi-Square test or Fisher's exact test. Means of SUA were compared across genotypes using one-way analysis of variance.

Results

In total, 235 Hmong adults participated in the study (Figure 1). Demographics and anthropometric measures are summarized in Table 1. Briefly, the mean ($\pm$ standard deviation [SD]) age of all study participants was 30.3 (± 15.7) years with a slight majority of females (55%). More than 60% of the participants had $\text{BMI} \geq 25 \text{ kg/m}^2$. Prevalence of self-reported gout was 5.1%. In addition, 57 participants agreed to have their SUA levels measured, with a mean ($\pm$ SD) SUA level of 6.3 (± 1.7) mg/dl. Of the 57 participants, 27 (47%) had SUA levels $\geq 6 \text{ mg/dl}$.

All the selected SNPs were in Hardy–Weinberg equilibrium with call rates $> 99.0\%$. Seven of the eight targeted SNPs were significantly different when comparing the prevalence between Hmong and CEU (Tables 2 & 3). Among the seven SNPs showing a difference, six of the risk alleles (rs505802 [C>T], rs2231142 [G>T], rs12129861 [G>A], rs11942223 [T>C], rs734553 [G>T] and rs1183201 [T>A]) were significantly more prevalent in the Hmong compared with the CEU population. However, the prevalence of the risk allele rs3733591 (A>G) was found to be significantly lower in the Hmong compared with CEU. For the comparisons between Hmong and CHB populations, three out of eight SNPs showed statistically significant differences. Two of the risk alleles were significantly more prevalent in the Hmong compared with CHB (rs3733591 [A>G] and rs1014290 [C>T]), and one risk allele rs12129861 (G>A) had lower prevalence. For the 57 adults with measured SUA levels, no significant associations between risk alleles and SUA levels or risk of hyperuricemia ($\text{SUA} \geq 6 \text{ mg/dl}$) were found. Association data analyses are not shown.

Discussion

This study identifies a statistically higher prevalence of validated risk alleles in our Hmong cohort compared with published data in CEU and CHB populations. Specifically, six out of eight risk alleles were more prevalent in the Hmong than CEU, and two out of three risk alleles were more prevalent in the Hmong than the CHB. This observation represents the first report of its kind, which may support a genetic basis for the notably higher prevalence of hyperuricemia and gout observed in the Hmong, relative to non-Hmong residing in Minnesota. Our findings of differences between Hmong and CHB population further support the importance of utilizing the more precise genomic-based guidance for risk associations rather than race or ethnic classifications. The roles that select genetic variations within genes (*SLC22A12*, *SLC2A9*, *ABCG2*, *SLC17A1* and *PDZK1*) affecting UA disposition play in

the manifestation of hyperuricemia, development of gout and modulating drug responsiveness are worthy of discussion.

SLC22A12 encodes for the URAT1 transporter found on the apical side of the proximal convoluted tubule in kidney. Loss-of-function mutations of URAT1 cause idiopathic renal hypouricemia due to high urinary urate excretion [35] and presumably a gain-of-function mutation could lead to hyperuricemia [36]. Considered a major carrier facilitating UA reabsorption, this transporter was deemed a logical target for urate-lowering therapies. The intergenic SNP rs505802 (C>T) with an estimated prevalence of the (T) allele at 73% was identified by Kolz *et al.* [17] to be associated with a lower SUA. By contrast, the prevalence of the (T) allele in our Hmong cohort was only 35% ($p < 0.001$) suggesting a higher risk for hyperuricemia in the Hmong, relative to CEUs. Incidentally, the estimated prevalence of the (T) allele in the CHB population was 26% which, although not significantly ($p = 0.0068$) lower compared with our Hmong cohort, suggests that the Hmong and CHB populations (both considered to be Asian) appear to display substantive differences in allele frequencies. Two other SNPs (rs893006 [A>G], and rs11231825 [C>T]) within *SLC22A12* were found to be significantly associated with elevated levels of SUA in a Japanese cohort, [20] or reduced FEUA in a German population [19]. Notably, both of these SNPs are in complete linkage disequilibrium ([LD] $r^2 = 1$) with the previously mentioned rs505802. Consequently, not only does rs505802 influence renal UA disposition, but it may also influence response to urate-lowering therapies. The basis for this argument resides in our understanding of the mechanism of action of two common urate-lowering therapies: probenecid and allopurinol. Both probenecid and more precisely, the active metabolite of allopurinol, known as oxipurinol, inhibit URAT1 by competing for reabsorption with UA [37,38]. Thus, our finding of a higher prevalence of the risk allele associated with hyperuricemia (rs505802 C>T) may not only partially explain the higher prevalence of gout in the Hmong, but also provide a basis for anecdotal reports questioning the effectiveness of allopurinol when used in the Hmong [28]. Genotype-based differences on renal handling of UA and the effectiveness of urate-lowering therapies complicate the interpretation of risk for hyperuricemia and response to select medications. Studies that decouple these competing factors should elucidate our understanding of the potential basis for genotype-guided selection of drug therapy.

SLC2A9 encodes for GLUT9 transporter, which is a high capacity transporter for fructose, glucose and UA. GLUT9 is not only expressed in the kidney and liver, but it is also expressed in the chondrocyte of human articular cartilage [39]. The latter maybe an important contributing factor for the deposition of monosodium urate crystals within and around joints affected with gout. The intronic SNP rs734553 (G>T) was shown to be significantly associated with increased SUA in the CEU population with an estimated prevalence of the (T) allele at 74% [17]. In our Hmong cohort, the estimated prevalence of the (T) allele was 99%, which was significantly higher than CEU ($p < 0.0001$). In this case, the prevalence of the (T) allele was not different between the Hmong and CHB, both at ~99% ($p = 0.269$). Once again, the Hmong's genetic predisposition to hyperuricemia is enhanced relative to CEU. Notably, the SNP rs734553 (G>T) is in moderate LD ($r^2 = 0.8$) with the intronic SNP rs11942223 (T>C) for which the (C) allele has been shown to be

protective against hyperuricemia [32]. The prevalence of the protective (C) allele in our Hmong cohort (1.3%) was again lower compared with CEU (25%; $p < 0.001$).

Also within *SLC2A9*, the nonsynonymous SNP rs3733591 (Arg265His) has been consistently associated with gout. For instance, Tu *et al.* [33] identified that a copy of the risk allele (C) had a higher risk for tophi with an odds ratio (OR) of 2.05 (95% CI: 1.11–3.77; $p = 0.0176$) in the CHB population while Urano *et al.* [40] demonstrated an increased risk for gout in Japanese with an OR of 1.52 (95% CI: 1.19–1.95; $p = 7.3 \times 10^{-4}$). In our Hmong cohort, the risk allele rs3733591 (A>G) is actually lower (41%) compared with CEU (80%; $p < 0.001$), but higher than CHB (30.7%; $p = 0.005$), further suggesting that the Hmong is a unique ethnic group in spite of being commonly classified as CHB.

Reduced FEUA is common in patients with gout and is a risk factor for developing hyperuricemia. The intronic SNP rs1014290 (C>T) within *SLC2A9* identified by Vitart *et al.* [31] was found to be significantly associated with decreased FEUA and development of gout in multiple populations of CEU descent. The presence of the (T) allele was significantly associated with OR (1.57 and 1.40) for reduced FEUA and gout, respectively. In our present study, the prevalence of (T) allele in our Hmong population (69.5%) was lower than CEU (74.3%; $p = 0.19$), but higher than CHB (59.6%; $p = 0.006$). This further suggests that decreased FEUA may be a contributing mechanism for developing gout in the Hmong which, again, differs from the CHB.

The *SLC17A1* gene encodes the voltage-gated cotransporter protein NPT1 on the apical membrane of the renal proximal tubule. Chiba *et al.* [41] have recently identified the putative mechanism and role by which NPT1 affects UA suggesting that NPT1 functions as a renal urate excreter. In a Japanese case–control study, these same investigators also identified the missense SNP rs1165196 (I269T) of *SLC17A1* gene to be a gain-of-function change which could protect against renal urate underexcretion. Kolz *et al.* [17] identified the intronic SNP rs1183201 (T>A) of the *SLC17A1* gene to be significantly associated with decreased serum UA with the effect allele (A) being the protective allele in CEU. In addition, this SNP was also found within a larger region on chromosome 6 suggesting its high LD with other SNPs. This finding could explain the moderate LD ($r^2 = 0.868$) between these two SNPs (rs1165196 and rs1183201) in CEU and strong LD (0.967) within CHB. Zhou *et al.* [42] have successfully replicated similar findings in CHB males case–control study by showing that SNP rs1183201 was significantly associated with gout and SUA and in consistency with Kolz *et al.* findings [17].

In our Hmong cohort, the frequency of the effect allele (A) for rs1183201 was threefold lower than that observed in Caucasians (16.4 vs 50%; $p < 0.001$). However, the frequency of the effect allele did not differ between Hmong and CHB (16.4 vs 13.3%; $p = 0.54$). The extensive validation of this SNP (rs1183201) across different ethnic groups and its direct role on renal UA excretion highlights a plausible mechanism for the Hmong to be at higher risk for developing gout, relative to other populations. These consistent findings in part, provide possible genetic basis for the differential prevalence of gout in Hmong relative to non-Hmong living in Minnesota [16].

ABCG2 encodes for the superfamily of ATP-binding cassette protein, which is a xenobiotic efflux transporter and major carrier for endogenous substances. Genetic polymorphisms in *ABCG2* have been associated with SUA and gout in select populations [43–45]. The missense SNP (rs2231142) Gln141Lys (G>T) has been shown to be associated with elevated SUA in CEU men [17] and indeed, the prevalence of the risk allele (T) in our Hmong cohort (36%) was higher than that in the CEU population (11%; $p < 0.001$). Significantly, this SNP (rs2231142) has also been strongly associated with response to allopurinol in white non-Hispanic and Hispanic populations [46]. This observation further strengthens the argument that genetic variability of transporters is associated with disposition of UA and could also modulate drug response.

The scaffolding structural protein PDZK1's role on UA disposition was evident from the meta-analysis conducted by Kolz *et al.* [17] who showed that the intergenic SNP rs12129861 (G>A) was significantly associated with lower SUA levels. This protein is essential for UA transporter activity including URAT1, OAT4 and NPT1 [47]. The prevalence of the (A) allele for rs12129861 (G>A), associated with decreased SUA, in our Hmong cohort (33%) was lower than CEU (46%; $p < 0.001$), but was higher than CHB (20%; $p = 0.002$). This finding is consistent with the elevated risk of gout in the Hmong relative to CEU while being supportive of our premise that there are differences between Hmong and CHB.

Finally, while different allele frequencies in Hmong could owe to their unique population history rather than a genuine causal effect on hyperuricemia or gout, we believe that the extensive validation of these selected SNPs across different population groups and ethnicities provides the basis to theorize that the different allele frequencies in the Hmong may be contributing to their enhanced risk of gout compared with CEU.

In general, advancing age increases the risk of developing gout due to a number of factors including declining kidney function, increased dehydration, increased use of drugs and increased prevalence of other comorbidities. The estimated prevalence of gout in our Hmong cohort was 5.1%, which is close to the 6.2% reported by Portis *et al.* [16] and notably higher than the overall US prevalence of 3.9% [3]. Our findings may provide evidence to support a genetic component to the clinical observations of a higher prevalence of gout, hyperuricemia and UA kidney stones [16,29] and reported suboptimal use of allopurinol [28] in Minnesota Hmong compared with non-Hmong. The consistency of urate transporter risk alleles within our Hmong cohort compared with other populations is at the very least suggestive of a genetic component to their elevated risk. However, several limitations of our study require further discussion.

Limitations

The key limitations of our study include those common to investigations of complex questions within minority populations. In our example, the Hmong are a minority refugee population in which to conduct research, especially involving the collection of DNA or blood, is challenging. However, working with the Hmong Genomics Board and translators as partners in research, ensured that participants were appropriately informed as to the nature and purpose of the study. Another limitation of our study relates to sample size. Although

the total sample size (n = 235) represents a reasonable basis to estimate the prevalence of the eight targeted SNPs, the modest sample size of individuals with measured SUA (n = 57) and gout (n = 12) limits the power of our analysis to determine an association between the genotypes tested, and risk of hyperuricemia or gout. Similarly, our sample size precluded meaningful analyses of these associations based on a sex-specific definitions of hyperuricemia that otherwise may be more appropriate than our clinically oriented cut off for hyperuricemia of SUA ≥ 6 mg/dl. We cannot exclude the possibility of a selection bias in that those who volunteered to have their SUA measured may differ from those who did not volunteer. A larger and appropriately powered study will be needed to ascertain the effect of these select SNPs on SUA levels and the presence of gout. Although some investigators have found an association with SUA and gout with other genes (*GCKR* and *LRRC16A*) [17,42,48], these genes were not evaluated in our population. Further investigations of direct associations with these genes and key other SNPs are worthy of direct study in this unique population. Finally, we did not have comprehensive information for concomitant use of drugs or dietary habits, both of which may have influenced SUA levels. Indeed, diet is an important determinant of SUA levels and is cited as a key common risk factor in those populations exhibiting elevated prevalence of gout and hyperuricemia [49]. Any additional study will need to explore dietary variations and its association with gout.

Conclusion

Hyperuricemia risk alleles are more prevalent in the Hmong than CEU and CHB, and are directionally consistent with the increased risk of hyperuricemia and gout when compared with either CEU or CHB populations. Although the mean age in our Hmong cohort was significantly lower relative to previous reports and we had a preponderance of female subjects, the Hmong in this study remain to exhibit a higher prevalence of self-reported gout compared with the US population. Given the extensive work by others in replicating the role of key SNPs discovered in CEUs to be comparable in magnitude and identical in direction in Indians, African-Americans and Japanese, we believe that our findings have advanced our knowledge of the risk for hyperuricemia and gout in the Hmong.

Future perspective

If replicated, the translational implications of our findings may lead to genomic-based differentiation of prevention and treatment strategies for individuals with specific genetic profiles. The consideration of genetic factors in the selection of therapies may also lead to improved patient outcomes and lower healthcare costs. Comparative efficacy studies utilizing genetic information have the potential to improve pharmacotherapeutic outcomes by avoiding unnecessary trials of less efficacious drug therapies. Ultimately, this genetic information may advance our capacity to apply principles of precision medicine to achieve improved outcomes. In addition, individual's genotype may ultimately replace the imprecise descriptors such as race when considering individual's risk for gout and/or response to gout therapy.

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References

Papers of special note have been highlighted as:

• of interest

1. Rho YH, Zhu Y, Choi HK. The epidemiology of uric acid and fructose. *Semin Nephrol.* 2011; 31(5): 410–419. [PubMed: 22000647]
2. Zhu Y, Pandya BJ, Choi HK. Comorbidities of gout and hyperuricemia in the US general population: NHANES 2007–2008. *Am J Med.* 2012; 125(7):679–687. [PubMed: 22626509]
3. Zhu Y, Pandya BJ, Choi HK. Prevalence of gout and hyperuricemia in the US general population: the National Health and Nutrition Examination Survey 2007–2008. *Arthritis Rheum.* 2011; 63(10): 3136–3141. [PubMed: 21800283]
4. George RL, Keenan RT. Genetics of hyperuricemia and gout: implications for the present and future. *Curr Rheumatol Rep.* 2013; 15(2):309. [PubMed: 23307580]
5. Alvarez-Lario B, Macarron-Vicente J. Uric acid and evolution. *Rheumatology (Oxford).* 2010; 49(11):2010–2015. [PubMed: 20627967]
6. Khanna D, Fitzgerald JD, Khanna PP, et al. 2012 American College of Rheumatology guidelines for management of gout. Part 1: systematic nonpharmacologic and pharmacologic therapeutic approaches to hyperuricemia. *Arthritis Care Res (Hoboken).* 2012; 64(10):1431–1446. [PubMed: 23024028]
7. Panoulas VF, Milionis HJ, Douglas KM, et al. Association of serum uric acid with cardiovascular disease in rheumatoid arthritis. *Rheumatology (Oxford).* 2007; 46(9):1466–1470. [PubMed: 17644822]
8. Feig DI. Hyperuricemia and hypertension. *Adv Chronic Kidney Dis.* 2012; 19(6):377–385. [PubMed: 23089272]
9. Sluijs I, Beulens JW, van der A DL, Spijkerman AM, Schulze MB, van der Schouw YT. Plasma uric acid is associated with increased risk of Type 2 diabetes independent of diet and metabolic risk factors. *J Nutr.* 2013; 143(1):80–85. [PubMed: 23173177]
10. Doherty M. New insights into the epidemiology of gout. *Rheumatology (Oxford).* 2009; 48(Suppl 2):ii2–ii8. [PubMed: 19447779]
11. Edwards NL. The role of hyperuricemia and gout in kidney and cardiovascular disease. *Cleve Clin J Med.* 2008; 75(Suppl 5):S13–S16.
- 12•. Singh JA. Racial and gender disparities among patients with gout. *Curr Rheumatol Rep.* 2013; 15(2):307. A very concise review that summarizes the prevalence of gout across different races while highlighting some of the genetic and nongenetic factors that could contribute to such a large variability in gout prevalence. [PubMed: 23315156]
13. Charles BA, Shriner D, Doumatey A, et al. A genome-wide association study of serum uric acid in African Americans. *BMC Med Genomics.* 2011; 4:17. [PubMed: 21294900]
14. Klemp P, Stansfield SA, Castle B, Robertson MC. Gout is on the increase in New Zealand. *Ann Rheum Dis.* 1997; 56(1):22–26. [PubMed: 9059136]
15. Hakoda M. Recent trends in hyperuricemia and gout in Japan. *Japan Med Assoc J.* 2012; 55(4): 319–323.

- 16•. Portis AJ, Laliberte M, Tatman P, et al. High prevalence of gouty arthritis among the Hmong population in Minnesota. *Arthritis Care Res (Hoboken)*. 2010; 62(10):1386–1391. The first report to estimate gout prevalence among the Minnesota Hmong using self report of gout and retrospective chart review, which were compared with the overall prevalence of gout across the nation. [PubMed: 20506247]
- 17•. Kolz M, Johnson T, Sanna S, et al. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. *PLoS Genet*. 2009; 5(6):e1000504. A key meta-analysis of genome-wide association studies that was conducted on primarily Caucasian individuals numbering 28,141. The study identified nine genetic loci to be significantly associated with serum uric acid concentration. [PubMed: 19503597]
18. Kottgen A, Albrecht E, Teumer A, et al. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat Genet*. 2013; 45(2):145–154. [PubMed: 23263486]
19. Graessler J, Graessler A, Unger S, et al. Association of the human urate transporter 1 with reduced renal uric acid excretion and hyperuricemia in a German Caucasian population. *Arthritis Rheum*. 2006; 54(1):292–300. [PubMed: 16385546]
20. Shima Y, Teruya K, Ohta H. Association between intronic SNP in urate-anion exchanger gene, *SLC22A12*, and serum uric acid levels in Japanese. *Life Sci*. 2006; 79(23):2234–2237. [PubMed: 16920156]
21. Phipps-Green AJ, Hollis-Moffatt JE, Dalbeth N, et al. A strong role for the *ABCG2* gene in susceptibility to gout in New Zealand Pacific Island and Caucasian, but not Maori, case and control sample sets. *Hum Mol Genet*. 2010; 19(24):4813–4819. [PubMed: 20858603]
22. Hollis-Moffatt JE, Xu X, Dalbeth N, et al. Role of the urate transporter *SLC2A9* gene in susceptibility to gout in New Zealand Maori, Pacific Island, and Caucasian case-control sample sets. *Arthritis Rheum*. 2009; 60(11):3485–3492. [PubMed: 19877038]
23. Jang WC, Nam YH, Park SM, et al. T6092C polymorphism of *SLC22A12* gene is associated with serum uric acid concentrations in Korean male subjects. *Clin Chim Acta*. 2008; 398(1–2):140–144. [PubMed: 18824160]
24. Reginato AM, Mount DB, Yang I, Choi HK. The genetics of hyperuricaemia and gout. *Nat Rev Rheumatol*. 2012; 8(10):610–621. [PubMed: 22945592]
25. Beer NL, Tribble ND, McCulloch LJ, et al. The P446L variant in *GCKR* associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. *Hum Mol Genet*. 2009; 18(21):4081–4088. [PubMed: 19643913]
26. Tang L, Ye H, Hong Q, et al. Elevated CpG island methylation of *GCK* gene predicts the risk of Type 2 diabetes in Chinese males. *Gene*. 2014; 547(2):329–333. [PubMed: 24992032]
27. United States Census Bureau. The Asian Population: 2010. US Department of Commerce, Economics and Statistics Administration; WA, DC, USA: 2012. p. 23
- 28•. Wahedduddin S, Singh JA, Culhane-Pera KA, Gertner E. Gout in the Hmong in the United States. *J Clin Rheumatol*. 2010; 16(6):262–266. A retrospective study report compared the characteristics of gout in Hmong patients versus Caucasians and examined if Hmong ethnicity is associated with risk of tophaceous gout. The Hmong ethnicity alone was associated with higher risk of gout and Hmong patients with gout tend to discontinue allopurinol earlier than non-Hmong. [PubMed: 20808166]
- 29•. Portis AJ, Hermans K, Culhane-Pera KA, Curhan GC. Stone disease in the Hmong of Minnesota: initial description of a high-risk population. *J Endourol*. 2004; 18(9):853–857. An important report on the prevalence and complications of gout comorbidities associated with Hmong patients with gout. [PubMed: 15659918]
30. Israel, BA. *Methods in Community-based Participatory Research for Health*. 1st. Jossey-Bass; CA, USA: 2005.
31. Vitart V, Rudan I, Hayward C, et al. *SLC2A9* is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. *Nat Genet*. 2008; 40(4):437–442. [PubMed: 18327257]

32. Dalbeth N, House ME, Gamble GD, et al. Population-specific influence of *SLC2A9* genotype on the acute hyperuricaemic response to a fructose load. *Ann Rheum Dis.* 2013; 72(11):1868–1873. [PubMed: 23349133]
33. Tu HP, Chen CJ, Tovosia S, et al. Associations of a non-synonymous variant in *SLC2A9* with gouty arthritis and uric acid levels in Han Chinese subjects and Solomon Islanders. *Ann Rheum Dis.* 2010; 69(5):887–890. [PubMed: 19723617]
34. International HapMap Project. HapMap Genome Browser, release #28. 2010; 2014 www.hapmap.org/.
35. Hamada T, Ichida K, Hosoyamada M, et al. Uricosuric action of losartan via the inhibition of urate transporter 1 (URAT 1) in hypertensive patients. *Am J Hypertens.* 2008; 21(10):1157–1162. [PubMed: 18670416]
36. Kamper AL, Nielsen AH. Uricosuric effect of losartan in patients with renal transplants. *Transplantation.* 2001; 72(4):671–674. [PubMed: 11544429]
37. Stocker SL, Williams KM, McLachlan AJ, Graham GG, Day RO. Pharmacokinetic and pharmacodynamic interaction between allopurinol and probenecid in healthy subjects. *Clin Pharmacokinet.* 2008; 47(2):111–118. [PubMed: 18193917]
38. Stocker SL, Graham GG, McLachlan AJ, Williams KM, Day RO. Pharmacokinetic and pharmacodynamic interaction between allopurinol and probenecid in patients with gout. *J Rheumatol.* 2011; 38(5):904–910. [PubMed: 21285173]
39. Mobasheri A, Neama G, Bell S, Richardson S, Carter SD. Human articular chondrocytes express three facilitative glucose transporter isoforms: GLUT1, GLUT3 and GLUT9. *Cell Biol Int.* 2002; 26(3):297–300. [PubMed: 11991658]
40. Urano W, Taniguchi A, Anzai N, et al. Association between GLUT9 and gout in Japanese men. *Ann Rheum Dis.* 2010; 69(5):932–933. [PubMed: 20413573]
41. Chiba T, Matsuo H, Kawamura Y, et al. NPT1/SLC17A1 is a renal urate exporter in humans and its common gain-of-function variant decreases the risk of renal underexcretion gout. *Arthritis Rheumatol.* 2015; 67(1):281–287. [PubMed: 25252215]
42. Zhou ZW, Cui LL, Han L, et al. Polymorphisms in *GCKR*, *SLC17A1* and *SLC22A12* were associated with phenotype gout in Han Chinese males: a case–control study. *BMC Med Genet.* 2015; 16:66. [PubMed: 26290326]
43. Matsuo H, Takada T, Ichida K, et al. Common defects of *ABCG2*, a high-capacity urate exporter, cause gout: a function-based genetic analysis in a Japanese population. *Sci Transl Med.* 2009; 1(5):5ra11.
44. Wang B, Miao Z, Liu S, et al. Genetic analysis of *ABCG2* gene C421A polymorphism with gout disease in Chinese Han male population. *Hum Genet.* 2010; 127(2):245–246. [PubMed: 19921266]
45. Yamagishi K, Tanigawa T, Kitamura A, et al. The rs2231142 variant of the *ABCG2* gene is associated with uric acid levels and gout among Japanese people. *Rheumatology (Oxford).* 2010; 49(8):1461–1465. [PubMed: 20421215]
46. Wen CC, Yee SW, Liang X, et al. Genome-wide association study identifies *ABCG2* (BCRP) as an allopurinol transporter and a determinant of drug response. *Clin Pharmacol Ther.* 2015; 97(5): 518–525. The first genome-wide association study that attempted to indentify SNPs associated with variability of response to allopurinol. [PubMed: 25676789]
47. Wright AF, Rudan I, Hastie ND, Campbell H. A ‘complexity’ of urate transporters. *Kidney Int.* 2010; 78(5):446–452. [PubMed: 20613716]
48. Sakiyama M, Matsuo H, Shimizu S, et al. Common variant of leucine-rich repeat-containing 16A (*LRRC16A*) gene is associated with gout susceptibility. *Hum Cell.* 2014; 27(1):1–4. [PubMed: 24318514]
49. Wang Y, Yan S, Li C, et al. Risk factors for gout developed from hyperuricemia in China: a five-year prospective cohort study. *Rheumatol Int.* 2013; 33(3):705–710. [PubMed: 22544037]

Executive summary

Background

- Hyperuricemia, or high serum uric acid (SUA) level, is the strongest predictor for developing gout.
- Gout and hyperuricemia have been strongly associated with cardiovascular diseases, Type 2 diabetes, kidney disease and ureteral kidney stones.
- The Hmong are unique ethnic groups that have a two- to five-fold higher risks for gout and gout-related comorbidities.
- Genetic risk factors of hyperuricemia and gout have been explored in select populations but not the Hmong.

Patients & methods

- In total, 235 Hmong of which 55% were females with mean ($\pm$ standard deviation) age of 30.3 years ($\pm$ 15.7), who were recruited from various community and clinic settings and provided salivary DNA.
- Eight SNPs within five key candidate genes (*SLC22A12*, *SLC2A9*, *ABCG2*, *SLC17A1* and *PDZK1*) were genotyped using either iPLEX gold method or TaqMan.
- Frequencies of risk alleles associated with hyperuricemia were calculated in the Hmong and compared with two distinct reference populations: European (CEU) and Han-Chinese (CHB). SUA was measured for select participants (n = 57).

Results

- The frequencies of seven out of eight risk alleles in the Hmong was significantly different than CEU. Six risk alleles (rs505802 [C>T], rs2231142 [G>T], rs12129861 [G>A], rs11942223 [T>C], rs734553 [T>G], rs1183201 [T>A]) had significantly higher prevalence while one (rs3733591 [T>C]) had a lower prevalence.
- The frequencies of three out of seven risk alleles in the Hmong were significantly different than CHB. Two risk alleles (rs3733591 [T>C], rs1014290 [A>G]) had significantly higher prevalence while one (rs12129861 [G>A]) had a lower prevalence.
- SNP by SNP analysis did not show clear association with SUA levels.
- The prevalence of self-reported gout was 5.1%.

Conclusion

- The frequency of hyperuricemia risk alleles of select genes in the Hmong was higher in either CEU or CHB populations which may, in part, explain their higher risk for developing gout.

Future perspective

- The higher prevalence of hyperuricemia risk alleles in the Hmong versus CEU and CHB populations may not just explain their higher risk for gout but may also impact their response to urate-lowering therapies.

Figure 1. Risk of hyperuricemia[†] and self-reported gout in 235 participants

Distribution of individuals among those with unknown or measured SUA by self-reported history of gout.

[†]Risk for hyperuricemia was defined as SUA ≥ 6 mg/dl.

SUA: Serum uric acid.

Table 1

Clinical characteristics and demographics of study participants (n = 235).

Participant characteristics	n (%) or mean $\pm$ SD	Minimum	Maximum
Age (years)	30.3 $\pm$ 15.7	18	87
Sex:			
– Females	130 (55.3)		
– Males	105 (44.7)		
Smoking status:			
– Never smoked	191 (81.3)		
– Former smoker	20 (8.5)		
– Current smoker	20 (7.2)		
– Missing data	7 (3.0)		
Self-reported medical history:			
– Type 2 diabetes	31 (13.2)		
– Hypertension	15 (6.4)		
– Hyperlipidemia	14 (6.0)		
– Gout	12 (5.1)		
– Kidney stone	6 (2.6)		
BMI (kg/m ²)	27.8 $\pm$ 6.2	16.7	52.1
SUA (mg/dl) (n = 57)	6.3 $\pm$ 1.7	3.5	11.4
Females SUA (mg/dl) (n = 39)	5.9 $\pm$ 1.6	3.5	9.9
Males SUA (mg/dl) (n = 18)	7.1 $\pm$ 1.6	4.8	11.4
Waist circumference inches/(cm):			
– Females	33.3 $\pm$ 5.7/(84.5 $\pm$ 14.4)	24.5/(62)	50.0/(127)
– Males	35.6 $\pm$ 5.4/(90.4 $\pm$ 13.6)	26.0/(66)	51.5/(131)
Systolic blood pressure (mmHg)	117 $\pm$ 16	80	178
Diastolic blood pressure (mmHg)	72 $\pm$ 10	40	101
Self-reported years in USA (years)	15.6 $\pm$ 8.7	3	31

SD: Standard deviation; SUA: Serum uric acid.

Table 2

Alleles frequencies within Hmong, European and Han-Chinese.

Gene (SNP) [†]	Allele	Hmong, n (%)	CEU, n (%)	p-value Hmong vs CEU	CHB, n (%)	p-value Hmong vs CHB
<i>SLC22A12</i> (rs505802)						
	C	297 (64.8)	62 (27.4)	<0.001	204 (74.5)	0.0068
	T	161 (35.2)	164 (72.6)		70 (25.5)	
	Total	458	226		274	
<i>SLC2A9</i> (rs3733591)						
	A	265 (58.6)	43 (19.7)	<0.001	190 (69.3)	0.005
	G	187 (41.4)	175 (80.3)		84 (30.7)	
	Total	452	218		274	
<i>SLC2A9</i> (rs11942223)						
	T	452 (98.7)	180 (75.0)	<0.001	178 (98.9)	1
	C	6 (1.3)	60 (25.0)		2 (1.1)	
	Total	458	240		180	
<i>SLC2A9</i> (rs734553)						
	T	459 (98.5)	167 (73.9)	<0.001	173 (99.6)	0.269
	G	7 (15.0)	59 (26.1)		1 (0.4)	
	Total	466	226		174	
<i>SLC2A9</i> (rs1014290)						
	T	317 (69.5)	168 (74.3)	0.191	161 (59.6)	0.006
	C	139 (30.5)	58 (25.7)		109 (40.4)	
	Total	456	226		270	
<i>ABCG2</i> (rs2231142)						
	G	293 (63.7)	201 (88.9)	<0.001	194 (70.8)	0.059
	T	167 (36.3)	25 (11.1)		80 (29.2)	
	Total	460	226		274	
<i>SLC17A1</i> (rs1183201)						

Gene (SNP) [‡]	Allele	Hmong, n (%)	CEU, n (%)	p-value Hmong vs CEU	CHB, n (%)	p-value Hmong vs CHB
<i>PDZK1</i> (rs12129861)	A	75 (16.4)	63 (50)	<0.001	12 (13.3)	0.559
	T	381 (83.6)	63 (50)		78 (86.7)	
	Total	456	126		90	
	G	307 (67.3)	122 (53.9)	<0.001	220 (80.3)	0.002
	A	149 (32.7)	104 (46.1)		54 (19.7)	
	Total	456	226		274	

[‡] Bold letter allele indicates the risk allele, which is defined as the allele that is associated with higher serum uric acid or could adversely increase serum uric acid levels. Bold p-values highlight those meeting our definition of statistical significance.
CEU: European; CHB: Han-Chinese.

Table 3

Genotype frequencies comparisons between Hmong, European and Han-Chinese.

Gene (SNP)	Genotype	Hmong, n (%)	CEU, n (%)	p-value Hmong vs CEU	CHB, n (%)	p-value Hmong vs CHB
<i>SLC22A12</i> (rs505802)	CC	101 (44)	10 (9)	<0.001	76 (55.5)	<0.001
	CT	95 (41.5)	42 (37)		52 (38)	
	TT	33 (14.5)	61 (54)		9 (6.6)	
	Total	229	112		137	
<i>SLC2A9</i> (rs1014290)	TT	105 (46.0)	6 (5.3)	<0.001	23 (17.0)	<0.001
	TC	107 (46.9)	46 (40.7)		63 (46.7)	
	CC	16 (7.0)	61 (54.0)		49 (36.3)	
	Total	228	113		135	
<i>SLC2A9</i> (rs11942223)	TT	223 (97.4)	33 (50.8)	<0.001	44 (97.8)	0.999
	TC	6 (2.6)	31 (47.7)		1 (2.2)	
	CC	0 (0.0)	1 (1.5)		0 (0)	
	Total	229	65		45	
<i>SLC2A9</i> (rs734553)	TT	226 (97.0)	60 (53.1)	<0.001	136 (99.3)	0.266
	GT	7 (3.0)	47 (41.6)		1 (0.7)	
	GG	0	6 (5.3)		0	
	Total	233	113		137	
<i>SLC2A9</i> (rs7373591)	GG	38 (17)	71 (65)	<0.001	9 (7)	0.0077
	GA	111 (49)	33 (30)		66 (48)	
	AA	77 (34)	5 (5)		62 (45)	
	Total	226	109		137	
<i>ABCG2</i> (rs2231142)						

Gene (SNP)	Genotype	Hmong, n (%)	CEU, n (%)	p-value Hmong vs CEU	CHB, n (%)	p-value Hmong vs CHB
<i>SLC17A1</i> (rs1183201)	CC	96 (41.7)	89 (78.8)	<0.001	69 (50.4)	0.211
	CA	101 (43.9)	23 (20.4)		56 (40.9)	
	AA	33 (14.3)	1 (0.9)		12 (8.8)	
	Total	230	113		137	
	AA	7 (3.1)	17 (27)	<0.001	0	0.489
<i>PDZK1</i> (rs12129861)	AT	61 (26.8)	29 (46)	0.0039	12 (26.7)	
	TT	160 (70.2)	17 (27)		33 (73.3)	
	Total	228	63		45	
	GG	105 (46.1)	35 (31.0)		88 (64.2)	<0.001
	AG	97 (42.5)	52 (46.0)		44 (32.1)	
	AA	26 (11.4)	26 (23.0)		5 (3.6)	
	Total	228	113		137	

Bold p-values highlight those meeting our definition of statistical significance.

CEU: European; CHB: Han-Chinese.