Association of SLC2A9 genotype with phenotypic variability of serum urate in pre-menopausal women

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The SLC2A9 gene, that encodes a renal uric acid reuptake transporter, has genetic variants that explain ~3% of variance in urate levels. There are previous reports of non-additive interaction between SLC2A9 genotype and environmental factors which influence urate control. Therefore, our aim was to further investigate the general phenomenon that such non-additive interactions contribute to genotype-specific association with variance at SLC2A9. Data from 14135 European individuals were used in this analysis. The measure of variance was derived from a ranked inverse normal transformation of residuals obtained by regressing known urate-influencing factors (sex, age, and body mass index) against urate. Variant rs6449173 showed the most significant effect on serum urate variance at SLC2A9 ($P = 7.9 \times 10^{-14}$), which was maintained after accounting for the effect on average serum urate levels ($P = 0.022$). Noting the stronger effect in a sub-cohort that consisted of pre-menopausal women and younger men, the participants were stratified into males and pre-menopausal and post-menopausal women. This revealed a strong effect on variance in pre-menopausal women ($P = 3.7 \times 10^{-5}$) with a weak effect in post-menopausal women ($P = 0.032$) and no effect in men ($P = 0.22$). The T-allele of rs6449173, which associates with increased urate levels, was associated with the greater variance in urate. There was a non-additive interaction between rs6449173 genotype and female gender in control of serum urate levels that was driven by a greater increase in urate levels associated with the T-allele in women. Female hormones, and/or other factors they influence or are associated with (such as iron levels, temperature, testosterone) interact with SLC2A9 genotype in women to determine urate levels. The association of SLC2A9 with greater variance in pre-menopausal women may reflect the cyclical changes resulting from menstruation.

Keywords: genotype, exposure, interaction, urate, SLC2A9, variance, gout, uric acid

INTRODUCTION

Heterogeneity in genetic variance exists when the effect a genotype has on phenotype is influenced by external factors. Such factors include differing environmental exposures, internal factors (such as epistatic interactions with other genetic variants), or other biological phenomena. An example of the latter are the stochastic processes underlying photoreceptor choice of cone cells in developing tri-chromatic vision or increased variation with aging within individuals of a given genotype.
Urate is a medically important metabolite. Elevated serum urate (hyperuricemia) is a central cause of gout, the most common form of inflammatory arthritis characterized by severe pain, disability, and joint damage. A genome-wide association study (GWAS) has demonstrated that levels of serum urate are influenced by genetic variants in 28 loci, with the strongest effects observed in renal and gut transporters of uric acid (Köttgen et al., 2013). In particular, variants in SLC2A9 have a very large effect on urate levels (e.g., rs12498742) and gout [e.g., rs11942223; in strong linkage disequilibrium (LD) with rs12498742], explaining 2–3% of the variance in serum urate in European individuals and a substantially stronger effect in women than in men (Hollis-Moffatt et al., 2009; Köttgen et al., 2013). Sex is the strongest reported interacting variable with SLC2A9 genotype to control urate levels \( (P = 8.2 \times 10^{-6}) \) for sex, \( P = 0.02–0.03 \) for age and alcohol intake, \( P > 0.38 \) for BMI, diabetes and hypertension status (Voruganti et al., 2014).

In addition, non-additive interactions between SLC2A9 genetic variants, food items, and diuretic medication have been reported. The influence of diet and diuretic medication on serum urate is well-established. Use of diuretics and consumption of seafood, red meat, alcohol and sugar-sweetened beverage (SSB) and tomatoes all associate with increased urate and the risk of gout (Choi and Curhan, 2004, 2008; Choi et al., 2010), a single dimensional analysis for genotypes influencing phenotypic variance could be used. Therefore, the aim of this study was to test for association with variance in serum urate at SLC2A9 and potentially identify other environmental interactions with SLC2A9 in serum urate.

### MATERIALS AND METHODS

#### Participants

Participants of European ancestry were included from five separate sample sets (Table 1). Two were from the Atherosclerosis Risk in Communities study (ARIC; \( n = 3562 \)) and the Framingham Heart Study (FHS Generation 3; \( n = 3282 \)) from which people taking antihypertensive or urate-lowering

### TABLE 1 | Demographic and clinical details of the three data sets, and associations of clinical features with serum urate concentrations.

|          | FHS \( r^2 \)\(^\wedge\) | ARIC \( r^2 \) | ARIC diuretics \( r^2 \) | CARDIA \( r^2 \) | CHS \( r^2 \) |
|----------|----------------|-------------|------------------|-------------|-------------|
| Serum urate (SD, mmol/L) | 0.308 (0.086) | – | 0.332 (0.080) | – | 0.398 (0.097) | – | 0.284 (0.087) | – | 0.328 (0.087) | – |
| Age (SD), years | 39.4 (8.64) | 8.51 \( \times 10^{-5} \) | 53.5 (5.58) | 0.0062 | 55.9 (5.55) | 0.015 | 40.7 (3.33) | 4.55 \( \times 10^{-5} \) | 72.4 (54.7) | 4.75 \( \times 10^{-4} \) |
| Females, % (n) | 53.8 (1765) | 0.44 | 54.2 (2903) | 0.29 | 65.7 (798) | 0.15 | 53.7 (803) | 0.39 | 61.1 (1710) | 0.11 |
| BMI (SD), kg/m\(^2\) | 26.4 (5.18) | 0.16 | 26.0 (4.33) | 0.14 | 29.2 (5.71) | 0.070 | 27.1 (5.85) | 0.14 | 26.2 (4.42) | 0.088 |
| Post-menopausal women, % (n)* | 9.0 (159) | 0.0066 | 49.8 (1447) | 0.022 | 51.0 (401) | 0.017 | 5.2 (42) | 0.0050 | 89.4 (1528) | – |

*Excluding 90 FHS, 942 ARIC, 266 ARIC diuretic, 234 CARDIA, and 182 CHS participants for which menopausal status was unknown, intermediate or were receiving hormone replacement therapy. The \( R^2 \) value is the proportion of variance in urate explained by menopause in women with known menopause status.

\( \wedge R^2 \) values, representing the proportion of variance in serum urate explained by the variable, were obtained by linear regression of urate against the variable listed, using the lm function in R, and extracting the Multiple \( R^2 \) value from the output summary. SD, standard deviation; PC, principal component.
medication, or who self-reported physician-diagnosed kidney disease or gout were excluded. Two were from the Coronary Artery Risk Development in Young Adults study (CARDIA; \(n = 1496\)) and the Cardiovascular Health Study (CHS; \(n = 2799\)), from which individuals taking urate-lowering medication and who self-reported physician-diagnosed kidney disease or gout were excluded. ARIC individuals self-reporting as taking diuretics \((n = 1196)\) were also included as the fifth sample set. No individuals were excluded based on estimated glomerular filtration rate (eGFR) – there were 47 (0.33\%) individuals with eGFR < 30, 46 of whom were from CHS and one from ARIC. The research procedures were in accordance with the ethical standards of the institutional review boards relevant to the various data sets. Written informed consent was given by all participants. The ARIC, FHS, CHS, and CARDIA analyses (project #834) were approved by the relevant Database of Genotype and Phenotype1 Data Access Committees. The overall project was approved by the New Zealand Health and Disability Ethics Committee (ref: 05/10/130).

Phenotypes

Phenotypes from baseline exams were used for all studies with the exception of CARDIA, where phenotypes from exam six were used. For the total 7967 European female participants menopause status was determined by self-report. Those who were pregnant, breastfeeding, taking hormone replacement therapy, or did not report menopause status were excluded from the menopause analysis. Subjects who reported as post-menopausal, but had menstruated in the last 12 months were also excluded from the menopause analysis. Serum urate levels were measured using a standard uricase assay (precision value of 8.6\%) in the ARIC and CARDIA datasets (Henry et al., 1957; ARIC Investigators, 1989; Dyer et al., 1999). CHS used a Kodak Ektachem 700 analyzer with reagents (Eastman Kodak, Rochester, NY, USA), which had a coefficient of variation of 2.4\% (Cushman et al., 1995). A phosphotungstic acid reagent autoanalyzer was used to measure serum urate levels in the FHS data set participants (Crowley, 1964). This method has a precision value of 2.8\% (Henry et al., 1957; Crowley, 1964).

Genotypes

Publicly available genome-wide genotype data (Affymetrix 6.0) from the ARIC and CARDIA data sets, combined Affymetrix 50K and 500K platform data from the FHS data set and CHS genotypes imputed from Illumina Human CNV370v1 was used to impute the full \(SLC2A9\) region (±200 kb) using Impute2 version 2.3.0 with the 1000 Genomes Phase 1 integrated variant set phased with SHAPEIT2 as the reference haplotype panel (Delaneau et al., 2014).

Statistical Analysis

Analysis was done using the R statistical software package (version 3.2^2). R code is presented in Supplementary Table S1.

The variable used as a measure of variance was derived from residuals obtained from sex- and cohort-specific analysis

\(^2\text{http://www.R-project.org/}\)
regressing age and BMI (BMI causally affects urate levels (Lyngdoh et al., 2012; Palmer et al., 2013)). The top two principal component eigenvectors (calculated using default parameters with SMARTPCA (Patterson et al., 2006)) were also included to account for cryptic relatedness within sample sets. A ranked inverse normal transformation of the absolute residual values yielded the z-score, with the z2-score being the variance variable. The inverse normal transformation, while likely to be overly conservative, minimizes a possible mean-variance relationship of phenotype (Yang et al., 2012). To account for the influence of the mean effect of rs6449173 genotype on the variance effect, using the approach of Yang et al. (2012), the genotype-specific mean urate was subtracted from the urate level of each individual participant and the genotype effect on variance was retested on squared residuals as described above. Data sets were combined by inverse-variance weighted meta-analysis in R (meta version 4.2-01) using a fixed effects model, except where there was evidence for heterogeneity (P_{Het} < 0.05) whereupon a random effects model was used.

Interaction analysis between menopausal status and rs6449173 was conducted using the R lm function with a linear model regressing urate against age, BMI, rs6449173 allele, menopausal status and the interaction term between menopause and rs6449173 allele. Post-menopausal and pre-menopausal women were compared to men (as the referent group) in separate models and the effect of the interaction term reported.

### RESULTS

Analysis of all SLC2A9 variants within ±200 kb of the gene for association with variance in serum urate levels resulted in a single association peak (Figure 1). We chose to analyze SNP rs6449173 which is one of a large number (n = 136) of SNPs (Supplementary Figure S1) in a haplotype block including the variant (rs12498742, r2 = 0.96 with rs6449173) previously reported as most strongly associated with average urate by GWAS (Köttgen et al., 2013). Rs6449173 demonstrated the strongest effect on serum urate variance at this locus (Figure 1; Table 2; βT allele = −0.152, P = 7.9 × 10−14). The initial region-wide analysis (Figure 1) was unadjusted for the possible confounding effect of genotype-specific mean urate levels. After adjustment of the mean effect the genotype-specific effect on the variance was reduced in magnitude, and the direction of effect was reversed, with the major T allele associated with greater variance in urate (βT allele = 0.047, P = 0.022).

The adjusted variance effect was statistically significant only in the CARDIA data set (Table 2; β = 0.208, P = 7.3 × 10−4); all other European sample sets β ≤ 0.043, P ≥ 0.14). Noting that this sample set was comprised entirely of younger individuals (Table 1; men and predominantly pre-menopausal woman); noting that the effect of SLC2A9 on average urate levels is stronger in women (Köttgen et al., 2013); and noting the association of menopause with serum urate levels (Hak and Choi, 2008) we therefore reanalyzed the SLC2A9 genotype effect on variance in men and pre-menopausal and post-menopausal women separately. This revealed that the variance effect was stronger in pre-menopausal women in the combined sample set (Table 2; β = 0.191, P = 3.7 × 10−5) than post-menopausal women (β = 0.087, P = 0.032) or men (β = −0.038, P = 0.22). The variance effect was visualized using box plots (Figure 2). This showed that increased median z2-scores and increased standard deviation were observed with the TT-genotype and decreased median z2-scores and standard deviation were associated with the GG genotype in pre-menopausal women. This effect was less obvious in post-menopausal women and was not observed in men.

The hypothesis that there was non-additive interaction between genotype and female hormone status in determining serum urate (adjusting for age and BMI) was evaluated. Men and post-menopausal women have similar estrogen levels and estrogen has a similar paracrine role, not acting solely as an endocrine factor produced by the ovaries in each group (Khosla et al., 1998; Simpson and Davis, 2001). We therefore expected pre-menopausal women to have an interaction effect of greater magnitude than post-menopausal women (when both groups are compared to men), reflective of the variance results. However, allelic interaction terms were of approximately

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### Table 2 | The influence of accounting for the rs6449173 average effect on the estimated serum urate variance effect.

| Parameter       | N  | β* | SE  | P   |
|-----------------|----|----|-----|-----|
| ARIC            | 5356 |   |     |     |
| Mean effect     | 0.023 | 0.002 | 8.7 × 10−56 |
| Unadjusted variance effect | −0.153 | 0.033 | 4.1 × 10−6 |
| Adjusted variance effect | 0.024 | 0.033 | 0.47 |
| FHS             | 3282 |   |     |     |
| Mean effect     | 0.023 | 0.002 | 2.7 × 10−38 |
| Unadjusted variance effect | −0.192 | 0.042 | 5.8 × 10−6 |
| Adjusted variance effect | 0.063 | 0.043 | 0.14 |
| ARIC diuretics  | 1196 |   |     |     |
| Mean effect     | 0.031 | 0.004 | 2.5 × 10−12 |
| Unadjusted variance effect | −0.117 | 0.073 | 0.11 |
| Adjusted variance effect | 0.043 | 0.073 | 0.56 |
| CHS             | 2799 |   |     |     |
| Mean effect     | 0.023 | 0.003 | 2.0 × 10−19 |
| Unadjusted variance effect | −0.156 | 0.045 | 4.7 × 10−4 |
| Adjusted variance effect | −0.016 | 0.045 | 0.72 |
| CARDIA          | 1496 |   |     |     |
| Mean effect     | 0.026 | 0.003 | 2.6 × 10−22 |
| Unadjusted variance effect | −0.079 | 0.062 | 0.20 |
| Adjusted variance effect | 0.208 | 0.061 | 7.3 × 10−4 |
| Combined        | 14129 |   |     |     |
| Mean effect     | 0.024 | 0.00095 | 4.7 × 10−140 |
| Unadjusted variance effect | −0.152 | 0.020 | 7.9 × 10−14 |
| Adjusted variance effect | 0.047 | 0.020 | 0.022 |

*Mean effect units mmol/L urate; variance effect is estimate of allelic additive effect on z2.*
TABLE 3 | Association of rs6449173 genotype with serum urate variance in sample sets stratified into men, pre-menopausal women and post-menopausal women, with adjustment for rs6449173 mean effect.

|          | N   | Variance β* | SE  | P     | r² # |
|----------|-----|-------------|-----|-------|------|
| ARIC     |     |             |     |       |      |
| Total    | 5556| 0.024       | 0.033| 0.47  | 0.00010|
| Men      | 2453| −0.053      | 0.060| 0.28  | 0.00047|
| Pre-menopausal women | 515 | 0.188       | 0.105| 0.074 | 0.00624|
| Post-menopausal women | 1447 | 0.083       | 0.063| 0.19  | 0.00119|
| FHS      |     |             |     |       |      |
| Total    | 3282| 0.063       | 0.043| 0.14  | 0.00067|
| Men      | 1517| −0.008      | 0.063| 0.89  | 0.00001|
| Pre-menopausal women | 1513 | 0.165       | 0.062| 7.6 × 10⁻³ | 0.00470|
| Post-menopausal women | 159  | −0.015      | 0.187| 0.93  | 0.00004|
| ARIC diuretics |     |             |     |       |      |
| Total    | 1196| 0.043       | 0.073| 0.56  | 0.00029|
| Men      | 410 | −0.088      | 0.126| 0.49  | 0.00119|
| Pre-menopausal women | 90  | 0.430       | 0.271| 0.12  | 0.02811|
| Post-menopausal women | 401 | 0.102       | 0.128| 0.43  | 0.00159|
| CHS      |     |             |     |       |      |
| Total    | 2799| −0.016      | 0.045| 0.72  | 0.00005|
| Men      | 1089| −0.121      | 0.073| 0.097 | 0.00253|
| Pre-menopausal women | 0  | --         | --  | --    | --  |
| Post-menopausal women | 1528 | 0.093       | 0.062| 0.13  | 0.00150|
| CARDIA   |     |             |     |       |      |
| Total    | 1496| 0.208       | 0.061| 7.3 × 10⁻⁴ | 0.00762|
| Men      | 693 | 0.112       | 0.092| 0.23  | 0.00211|
| Pre-menopausal women | 527 | 0.231       | 0.100| 0.022 | 0.00997|
| Post-menopausal women | 42  | 0.269       | 0.369| 0.47  | 0.01304|
| Combined |     |             |     |       |      |
| Total    | 14129| 0.047      | 0.020| 0.022 | 0.00037|
| Men      | 6162| −0.038      | 0.031| 0.22  | 0.00024|
| Pre-menopausal women | 2645 | 0.191       | 0.046| 3.7 × 10⁻⁵ | 0.00637|
| Post-menopausal women | 3577 | 0.087       | 0.040| 0.032 | 0.00129|

*Variance effect is estimate of allelic additive effect on z²

# r² values, representing the proportion of variance in serum urate explained by rs6449173, were obtained by linear regression of the adjusted z² value from the output summary.

DISCUSSION

We present evidence that the SLC2A9 genotype associated with average serum urate levels also differentially associates with variance in urate levels in pre-menopausal women. This may reflect the cyclical changes resulting from menstruation. There was also non-additive interaction between sex and SLC2A9 in determining urate levels, replicating the findings of Voruganti et al. (2014). We interpret these findings to indicate that the intrinsic biological phenomenon of female hormones (which change upon menopause) and/or other factors that they directly affect (such as temperature, iron levels, testosterone) interact with SLC2A9 genotype in a non-additive fashion in women to determine urate levels. The effect of the rs6449173 T-allele in raising urate is greater in women.

Our data can be compared to the findings of Yang et al. (2012) who associated FTO/IRX3 with genotype-specific variance in BMI. This locus, like SLC2A9, has the strongest mean effect size on phenotype in the genome. At the FTO SNP rs7202116 the allelic effect on average phenotype did not contribute to the observed effect on variance, in contrast with SLC2A9 rs6449173 where the allelic effect on mean phenotype contributed considerably to the genotype-specific association with variance.
in phenotype. Stratifying the sample set in our study clarified the analysis and clearly showed a genotype-specific effect on variance in urate in pre-menopausal women after accounting for the average effect. While a number of changes occur throughout the menstrual cycle (e.g., iron levels, temperature, estrogen and progesterone levels) the factors with the most evidence supporting a role in urate control are iron levels (Ghio et al., 2005; Mainous et al., 2011) and hormones. Female hormones (estrogens) increase the fractional excretion of uric acid and reduce serum urate levels (Yahyaoui et al., 2008). Our data are consistent with a model whereby female hormones contribute directly via \( \text{SLC2A9} \) in a genotype-specific fashion both to the mean urate levels and variance in urate levels. In animals estrogen reduces renal urate reabsorption by reducing \( \text{Slc2a9} \) protein levels (Takiue et al., 2011), so it is conceivable that in human females estrogen could contribute to the \( \text{SLC2A9} \)-mediated mean effect by a \( \text{rs6449173} \) genotype-specific effect on expression of \( \text{SLC2A9} \).

In pre-menopausal women urate levels vary across the menstrual cycle with endogenous estradiol associated with reduced, and follicle stimulating hormone associated with increased, urate (Mumford et al., 2013). Thus, also in a genotype specific manner, female hormones would be expected to contribute to variance potentially owing to the cyclical changes in levels of female hormones in pre-menopausal women or other factors influenced or associated with menstrual cycling in pre-menopausal women (e.g., oral contraceptive use Stöckl et al., 2012). Whilst estrogen levels in post-menopausal women are more similar to levels in men than pre-menopausal women (Khosla et al., 1998), and serum urate levels rise to levels approximately equivalent to those of men after menopause, the data in Table 4 suggest that post-menopausal women and men

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**FIGURE 2** | **Main effect adjusted \( z^2 \)-scores at \( \text{rs6449173} \).** The genotype-specific median (standard deviation) \( z^2 \) for men was GG 0.439 (1.458), GT 0.487 (1.443), TT 0.441 (1.380), for post-menopausal women was GG 0.411 (1.103), GT 0.465 (1.304), TT 0.451 (1.470), and for pre-menopausal women was GG 0.408 (0.891), GT 0.373 (1.215), TT 0.507 (1.514).

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**TABLE 4** | Association by linear regression and interaction of \( \text{rs6449173} \) genotype with average serum urate levels in pre-menopausal women and post-menopausal women and men stratified by genotype.

| \( \text{rs6449173} \) genotype | \( \text{GG} \) \( \beta \) (mmol/L), \( P \) | \( \text{GT} \) \( \beta \) (mmol/L), \( P \) | \( \text{TT} \) \( \beta \) (mmol/L), \( P \) | Interaction term \( \beta \) (mmol/L), \( P \) |
|-----------------------------|----------------|----------------|----------------|----------------|
| Men                         | 0.000          (Reference) | 0.021, \( 1.9 \times 10^{-4} \) | 0.038, \( 2.1 \times 10^{-15} \) | 0.000 (Reference) |
| Post-menopausal women       | \(-0.086, <2.0 \times 10^{-16} \) | \(-0.047, <2.0 \times 10^{-16} \) | \(-0.055, <2.0 \times 10^{-16} \) | \(0.012, 3.51 \times 10^{-6} \) |
| Pre-menopausal women        | \(-0.135, <2.0 \times 10^{-16} \) | \(-0.090, <2.0 \times 10^{-16} \) | \(-0.060, <2.0 \times 10^{-16} \) | \(0.013, 2.37 \times 10^{-6} \) |

Adjusted by age and BMI.
still control serum urate levels differently. However we were unable to test for a direct interaction between female hormone levels and SLC2A9 genotype. Owing to the use of cross-sectional data we were also unable to test for any genotype-specific effect on intra-individual variability in pre-menopausal women. Such a study would allow some evaluation of the hypothesis that SLC2A9 genotype interacts non-additively with female hormones or another variable factor associated with menstruation.

The association with variance was largely restricted to pre-menopausal women. There is epidemiological evidence from cross-sectional observational data that menopause associates (independent of measured confounders) with increased urate, that post-menopause hormone replacement therapy associates with reduced urate (Simon et al., 2006; Hak and Choi, 2008; Stöckl et al., 2012) and that estrogen levels are inversely associated with urate levels during the menstrual cycle (Mumford et al., 2013). This is consistent with clinical studies demonstrating a urate-lowering effect of hormone replacement therapy (Nicholls et al., 1973; Gottfredsen et al., 1983; Sumino et al., 1999), however, there is little definitive evidence that this effect occurs through an influence on renal uric acid handling (Nicholls et al., 1973; Gottfredsen et al., 1983; Antón et al., 1986; Ghio et al., 2005). The increased urate-associated TT genotype of rs6449173 drives the association with variance in urate in European pre-menopausal women (Figure 2), suggesting that understanding the molecular consequence of the genetic effect that this allele tags is key to understanding the mechanism for the observed genotype-specific effects of SLC2A9 on average urate and variability in urate. To this end, determining if rs6449173 is in fact associated with the separate SLC2A9 isoforms (full length and missing 28 cytoplasmic residues), as published data suggest (Döring et al., 2008; Vitart et al., 2008), will be important.

There are multiple independent effects at SLC2A9 with the urate association signal at SLC2A9 encompassing 100s of extremely strongly associated genetic variants over a very large region (500 kb; Kötting et al., 2013). In a GWAS of serum urate levels in East Asians (Okada et al., 2012), the strongest genome-wide association with urate was at SLC2A9, but with a different SNP variant (rs3775948). The most strongly associated European variant [rs12498742, in strong LD \( r^2 = 0.86 \) with rs6449173; Kötting et al., 2013] was not associated in the East Asian GWAS probably because of the rarity of the minor allele (prevalence of \( \sim 1\% \)). Interestingly the rs3775948 mean effect in East Asians also has, by conditional analysis, an effect in Europeans independent of the European mean effect (Stahl et al., 2014). Furthermore, a GWAS testing for association of common copy number variation with serum urate in Europeans (Scharpf et al., 2014) found association with two copy number variations 200 and 350 kb upstream of SLC2A9 that were each genetically independent of the rs12498742 effect at SLC2A9. Thus there is evidence for at least three independent variants in SLC2A9 that influence urate levels in Europeans, and a separate variant in East Asians. The study of Wei et al. (2014) is consistent with the above studies in providing evidence for multiple independent genetic effects at the SLC2A9 locus – five independent genetic effects were reported. Additional complexity in genetic control of urate levels at SLC2A9 was revealed with epistasis between genetic variants at the SLC2A9 locus influencing urate levels. [Note that rs6449173 and SNPs in strong LD were not amongst SNP pairs in Wei et al. (2014) exhibiting epistasis.] Combined with the evidence here for a genotype-dependent effect at SLC2A9 on variance, previous reports of non-additive GxE interaction at SLC2A9 (McAdams-DeMarco et al., 2013; Batt et al., 2014; Voruganti et al., 2014) and evidence for a population-specific influence of genotype to fructose response (Dalbeth et al., 2013), it is clear that this is an extremely complex urate and gout locus that will be very challenging to understand using genetic epidemiology.

The contribution of non-additive GxE interactions to the phenomenon of ‘missing’ heritability (predicted genetic variance not explained by genome-wide studies assessing the contribution of common genetic variants) is unclear, although it has been suggested that a failure to include the possibility of interactions in an inheritance model can lead to over-estimation of the genetic heritability of a phenotype (Manolio et al., 2009; Zuk et al., 2012). Urate levels are an ideal phenotype to address this question given that there are established dietary and drug environmental exposures (see Introduction) that have relatively immediate temporal effects on urate levels via hepatic production and perhaps also by interfering with excretion (Dalbeth and Merriman, 2013; Batt et al., 2014). This means that data on environmental exposures that are likely causal of changes in urate levels are able to be collected at the same time as phenotype in cross-sectional study designs. To facilitate identification of non-additive GxE interactions, systematically identifying genetic variants with a genotype-specific effect on variance in phenotype, in genome-wide approaches using very large sample sets and accounting for the average effect, can prioritize variants that can be tested for non-additive GxE with specific environmental exposures in linear and logistic models that incorporate interaction terms. Furthermore, identification of variance-associated genetic variants could allow identification of new urate loci which may have average main effects obscured in genome-wide studies that do not incorporate environmental exposures.

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Choi, H. K., Liu, S., and Curhan, G. (2005). Intake of purine-rich foods, protein, and red meat and risk of incident gout among patients with hypertension: a population based case-control study. *BMJ* 334, 8190. doi: 10.1136/bmj.d8190

Choi, H. K., and Curhan, G. (2008). Soft drinks, fructose consumption, and risk of incident gout among patients with hypertension: population based case-control study. *BMJ* 334, 8190. doi: 10.1136/bmj.d8190

Crowley, L. V. (1964). Determination of uric acid an automated analysis based on a carbonate method. *Clin. Chem.* 10, 838–844.

Cushman, M., Cornell, E. S., Howard, P. R., Bovill, E. G., and Tracy, R. P. (1995). Laboratory methods and quality assurance in the Cardiovascular Health Study. *Clin. Chem.* 41, 264–270.

Dalbeth, N., House, M. E., Gamble, G. D., Horne, A., Pool, B., Purvis, L., et al. (2013). Population-specific influence of SLC2A9 genotype on the acute hyperuricaemic response to a fructose load. *Ann. Rheum. Dis.* 72, 1868–1873. doi: 10.1136/annrheumdis-2012-202732

Dalbeth, N., and Merriman, T. (2013). “Hyperuricemia and gout,” in *The online metabolic and molecular bases of inherited disease (OMMBID)*, eds D. Valle, A. Beaudet, B. Vogelstein, K. Kinzler, S. Antonarakis, and A. Ballabio (New York, NY:McGraw-Hill Medical), 11, 106.

Delaneau, O., Marchini, J., and 1000 Genomes Project Consortium. (2014). Integrating sequence and array data to create an improved 1000 Genomes Project haplotype reference panel. *Nat. Commun.* 5:3934. doi: 10.1038/ncomms4934

Döring, A., Gieger, C., Mehta, D., Gohlik, H., Prokisch, H., and Coassin, S., et al. (2008). SLC2A9 influences uric acid concentrations with pronounced sex-specific effects. *Nat. Genet.* 40, 430–436. doi: 10.1038/ng.107

Dyer, A., Liu, K., Walsh, M., Kiefe, C., Jacobs, D. R. Jr., and Bild, D. E. (1999). Ten-year incidence of elevated blood pressure and its predictors: the CARDIA study. *Coronary Artery Risk Development in (Young) Adults. J. Hum. Hypertens.* 13, 13–21.

Flynn, T. J., Cadzow, M., Dalbeth, N., Jones, P. B., Stamp, I. K., Harré Hindmarsh, J., et al. (2015). Positive association of tomato consumption with serum urate: support for tomato consumption as an anecdotal trigger of gout flares. *BMC Musculoskelet. Disord.* 16:196. doi: 10.1186/s12891-015-0661-8

Geiler-Samerotte, K., Bauer, C., Li, S., Ziv, N., Gresham, D., and Siegal, M. (2013). Sex-specific effects of estrogen/gestagen therapy on uric acid metabolism in post-menopausal women. *Maturitas* 5, 9–15. doi: 10.1016/j.matur.2013.03.010

Ghio, A. J., Ford, E. S., Kennedy, T. P., and Hoidal, J. R. (2005). The association between serum ferritin and uric acid in humans. *Free Radic. Res.* 39, 337–342. doi: 10.1080/0100576040026088

Gottfredsen, A., Christiansen, C., and Transbol, I. (1983). Effect of natural oestrogen/gestagen therapy on uric acid metabolism in post-menopausal women. *Maturitas* 5, 9–15. doi: 10.1016/0378-5122(83)90016-8

Hak, A. E., and Choi, H. K. (2008). Menopause, postmenopausal hormone use and serum uric acid levels in US women—the Third National Health and Nutrition Examination Survey. *Arthritis Res. Ther.* 10:R116. doi: 10.1186/ar2519

Henry, R., Sobel, C., and Kim, J. (1957). A modified carbonate-phosphotungstate method for the determination of uric acid and comparison with...
the spectrophotometric uricase method. *Am. J. Clin. Pathol.* 28, 152–160.

Hollis-Moffatt, J. E., Xu, X., Dalbeth, N., Merriman, M. E., Topless, R., Wadell, C., et al. (2009). 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.* 60, 3485–3492. doi: 10.1002/art.24938

Investigators, A. R. I. C. (1989). The atherosclerosis risk in communities (ARIC) study: design and objectives. *Am. J. Epidemiol.* 129, 687–702.

Jacobs, G. H. (2009). Evolution of colour vision in mammals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 2957–2967. doi: 10.1098/rstb.2009.0039

Khosla, S., Melton, L. J. III, Atkinson, E. J., O’Fallon, W. M., Klee, G. G., and Riggs, B. L. (1998). Relationship of serum sex steroids and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. *J. Clin. Endocrinol. Metab.* 83, 2266–2274. doi: 10.1210/jc.83.7.2266

Köttgen, A., Albrecht, E., Teumer, A., Vitart, V., Krumnies, J., Hundermark, C., et al. (2013). Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat. Genet.* 45, 145–154. doi: 10.1038/n.2013.250

Lyngdoh, T., Vuistiner, P., Marques-Vidal, P., Rousson, V., Waeger, G., Vollenweider, P., et al. (2012). Serum uric acid and adiposity: deciphering causality using a bidirectional Mendelian randomization approach. *PLoS ONE* 7:e39321. doi: 10.1371/journal.pone.0039321

Mainous, A. G., Knoll, M. E., Everett, C. J., Matheson, E. M., Hulihan, M. M., and Grant, A. M. (2011). Uric acid as a potential cue to screen for iron overload. *Am. J. Epidemiol.* 211, 1410–1415. doi: 10.1093/aje/kwr129

Maroon, T. A., Halliwell, B., Stockley, P. A., and Jr., Hunter, D. J., et al. (2009). Finding the missing heritability of complex diseases. *Nature* 461, 747–753. doi: 10.1038/nature08494

McAdams-DeMarco, M. A., Maynard, J. W., Baer, A. N., Kao, L. W., Kottgen, A., Lyngdoh, T., Vuistiner, P., Marques-Vidal, P., Rousson, V., Waeger, G., Vollenweider, P., et al. (2012). Serum uric acid and adiposity: deciphering causality using a bidirectional Mendelian randomization approach. *PLoS ONE* 7:e39321. doi: 10.1371/journal.pone.0039321

Mainous, A. G., Knoll, M. E., Everett, C. J., Matheson, E. M., Hulihan, M. M., and Grant, A. M. (2011). Uric acid as a potential cue to screen for iron overload. *Am. J. Epidemiol.* 211, 1410–1415. doi: 10.1093/aje/kwr129

Maroon, T. A., Halliwell, B., Stockley, P. A., and Jr., Hunter, D. J., et al. (2009). Finding the missing heritability of complex diseases. *Nature* 461, 747–753. doi: 10.1038/nature08494

McAdams-DeMarco, M. A., Maynard, J. W., Baer, A. N., Kao, L. W., Kottgen, A., and Coresh, J. (2013). A urine gene-by-diuretic interaction and gout risk in participants with hypertension: results from the ARIC study. *Ann. Rheum. Dis.* 72, 701–706. doi: 10.1136/annrheumdis-2011-201186

Mumford, S. L., Dasharathy, S. S., Pollack, A. Z., Perkins, N. J., Mattison, D. R., Cole, S. R., et al. (2013). Serum uric acid in relation to endogenous reproductive hormones during the menstrual cycle: findings from the BioCycle study. *Hum. Reprod.* 28, 1853–1862. doi: 10.1093/humrep/det085

Nicholls, A., Snith, M., and Scott, J. (1973). Effect of oestrogen therapy upon urate transport systems in the mouse kidney. *Nucleosides Nucleotides Nucleic Acids* 30, 113–119. doi: 10.1080/15257770.2010.551645

Vitart, V., Rudan, I., Hayward, C., Gray, N. K., Floyd, J., Palmer, C. N., et al. (2008). SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. *Nat. Genet.* 40, 437–442. doi: 10.1038/ng.106

Voruganti, V. S., Franceschini, N., Haack, K., Laston, S., MacCleery, J. W., Uman, J. S., et al. (2014). Replication of the effect of SLC2A9 genetic variation on serum uric acid levels in American Indians. *Eur. J. Hum. Genet.* 22, 938–943. doi: 10.1038/ejhg.2013.264

Wei, W. H., Guo, Y., Kindt, A. S., Merriman, T. R., Semple, C. A., Wang, K., et al. (2014). Abundant local interactions in the 4p16.1 region suggest functional mechanisms underlying SLC2A9 associations with human serum uric acid. *Hum. Mol. Genet.* 23, 5061–5068. doi: 10.1093/hmg/ddu227

Yahyauoi, R., Esteva, I., Haro-Mora, J. I., Almaraz, M. C., Morcillo, S., Rojo-Martinez, G., et al. (2008). Effect of long-term administration of cross-sex hormone therapy on serum and urinary uric acid in transsexual persons. *J. Clin. Endocrinol. Metab.* 93, 2230–2233. doi: 10.1210/jc.2007-0247

Yang, J., Loos, R. J., Powell, J. E., Medland, S. E., Spielotes, E. K., Chasman, D. I., et al. (2012). FTO genotype is associated with phenotypic variability of body mass index. *Nature* 490, 267–272. doi: 10.1038/nature11401

Zuk, O., Hechter, E., Sunyaev, S. R., and Lander, E. S. (2012). The mystery of missing heritability: genetic interactions create phantom heritability. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1193–1198. doi: 10.1073/pnas.1119675109

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