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Genome-Wide Association Study Identifies ABCG2 (BCRP) as an Allopurinol Transporter and a Determinant of Drug Response

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The first-line treatment of hyperuricemia, which causes gout, is allopurinol. The allopurinol response is highly variable, with many users failing to achieve target serum uric acid (SUA) levels. No genome-wide association study (GWAS) has examined the genetic factors affecting allopurinol effectiveness. Using 2,027 subjects in Kaiser Permanente’s Genetic Epidemiology Research on Adult Health and Aging (GERA) Cohort, we conducted a GWAS of allopurinol-related SUA reduction, first in the largest ethnic group, non-Hispanic white (NHW) subjects, and then in a stratified transethnic meta-analysis. ABCG2, encoding the efflux pump BCRP, was associated with SUA reduction in NHW subjects (P = 2 × 10⁻⁸), and a missense allele (rs2231142) was associated with a reduced response (P = 3 × 10⁻⁷) in the meta-analysis. Isotopic uptake studies in cells demonstrated that BCRP transports allopurinol and genetic variants in ABCG2 affect this transport. Collectively, this first GWAS of allopurinol response demonstrates that ABCG2 is a key determinant of response to the drug.

Gout is a painful condition caused by deposition of uric acid (UA) crystals in the joints. This leads to an inflammatory arthritis with joint pain and swelling. The main cause of gout is hyperuricemia, which can be influenced by many factors including genetics, diet, medications, and renal failure (UA is mainly eliminated by the kidney with much lower elimination by the intestine).1 Gout remains the most common form of inflammatory arthritis in Western countries, and is on the rise worldwide in countries such as China, New Zealand, and Taiwan, with a particularly high prevalence (~10%) in the aboriginal populations of these latter two countries.1,2 In the UK, a recent study found that the prevalence of gout had increased to 2.5% by 2012.3,4 Importantly, less than half of UK patients with gout are treated with UA-lowering drugs.4 If left untreated, hyperuricemia can lead to nephrolithiasis and nephropathy and has also been associated with an increased risk of cardiovascular events, notably heart failure.2

Currently, allopurinol is the first-line medication for gout prevention.5 Allopurinol is a purine analog that is metabolized into oxypurinol. Both compounds act by inhibiting xanthine oxidase, the enzyme that converts xanthine into UA, thereby lowering serum uric acid (SUA) levels through the inhibition of its formation.

While a number of genetic risk factors for gout and hyperuricemia have been well established through multiple large genome-wide association studies (GWAS) and meta-analyses,6,7 only a few GWAS have focused on allopurinol, and all of them have centered on identifying genetic influences on its rare, life-threatening skin toxicities.8,9 No GWAS for allopurinol-related SUA reduction have been performed despite the fact that only 42% of patients on allopurinol are estimated to achieve the recommended SUA target of ≤6 mg/dl.10 Here we describe the first pharmacogenomic GWAS of allopurinol to identify genetic factors for response to the drug in a large multiethnic cohort of patients with comprehensive electronic pharmacy, laboratory, and clinical records. As confirmation, we followed up with laboratory experiments to verify some of the associations observed in the GWAS and explore the mechanism through which they affect response.

RESULTS

Overall, 2,027 patients in the GERA cohort met study inclusion criteria (Table 1). The resulting group of subjects had an average
of measurement (observed for baseline SUA, cumulative dose, and dose at the time allopurinol-related SUA change, the strongest effects were arrays). Among the nongenetic factors associated with first analyzed the sample of 1,492 NHW that were run on the largest group (after adjusting for nongenetic factors, this variant could account rated with a poorer response. When compared to the residuals after adjusting for nongenetic factors, this variant could account for 1.1% of the unexplained variance in NHW. In light of the BCRP association, we also examined the results for other genes/SNPs previously reported to be associated with gout and/or baseline uric acid. None of these genes/SNPs were found to be associated with allopurinol response (Supplemental Table S2), suggesting a unique association with BCRP.

Table 2  Nongenetic factors associated with uric acid change in response to allopurinol

<table>
<thead>
<tr>
<th>Nongenetic factor</th>
<th>r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−0.010</td>
<td>0.65</td>
</tr>
<tr>
<td>Baseline SUA</td>
<td>−0.71</td>
<td>&lt;2.2x10−16</td>
</tr>
<tr>
<td>BMI</td>
<td>0.012</td>
<td>0.59</td>
</tr>
<tr>
<td>Dose</td>
<td>−0.086</td>
<td>0.00013</td>
</tr>
<tr>
<td>Cumulative dose</td>
<td>−0.18</td>
<td>2.6x10−16</td>
</tr>
<tr>
<td>First principal component</td>
<td>0.042</td>
<td>0.072</td>
</tr>
<tr>
<td>Gender</td>
<td>−0.033</td>
<td>0.14</td>
</tr>
<tr>
<td>Concomitant medications</td>
<td>0.019</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Correlations are calculated comparing the nongenetic factor to the change between the pre-SUA residual and post-SUA residual found after correcting the pre- and post-SUA values for gender, age, BMI, and concomitant medications.
The results of the meta-analysis across race/ethnicity groups using the imputed genotype data showed that many additional suggestive SNPs within the ABCG2 locus were associated with SUA change, at \( P \)-values less than \( 1.4 \times 10^{-7} \) that did not reach genome-wide significance (Figure 1b). The previous strongest associated SNP, rs10011796, weakened in the meta-analysis due to differing results in other ethnic groups (from \( P = 2.0 \times 10^{-8} \) to \( P = 6.9 \times 10^{-4} \)). However, the previously noted functional SNP rs2231142 had a consistent direction of effect in all ethnicities, and became more significant (from \( P = 1.9 \times 10^{-6} \) to \( P = 3.4 \times 10^{-7} \)) (Figure 1b, Supplemental Table S3).

To verify our results and determine the mechanism by which ABCG2 variants associate with allopurinol response, we tested the hypothesis that BCRP transports allopurinol and oxypurinol, and that rs2231142 affects transport. Although not necessarily the only causal SNP, we chose to focus on rs2231142 since it is a known functional SNP and its significance strengthened in the meta-analysis. We used stably transfected BCRP-expressing cells, and used a cytotoxicity assay with mitoxantrone, a known BCRP substrate, as a positive control. As expected, cells expressing BCRP reference were the most resistant, followed by those with the Q141K variant, and followed by empty vector (Figure 2). In cell accumulation studies, BCRP-expressing cells had significantly lower levels of allopurinol and oxypurinol in comparison to empty vector transfected cells, consistent with an efflux role for both compounds (Figure 3a,b). Furthermore, addition of a specific BCRP inhibitor, Ko-143, caused significantly greater drug accumulation in BCRP expressing cells (Figure 3a,b). When cells were transfected with the rs2231142 (Q141K) variant, a significantly higher accumulation of both allopurinol and oxypurinol occurred as compared with reference BCRP transfected cells, consistent with reduced BCRP function (Figure 3c). Allopurinol and oxypurinol were not inhibitors of BCRP-mediated efflux of the model substrate pitavastatin (Figure 3d), suggesting that the drugs do not inhibit uric acid secretion via BCRP.

**DISCUSSION**

Our finding of a significant effect of genetic variation in ABCG2, and specifically the amino acid substitution Q141K, provides an avenue for understanding the response to allopurinol. SNPs in ABCG2 that have been previously associated with baseline SUA in many studies in multiple ethnic groups. Because BCRP is known to transport UA and various synthetic and naturally...
Table 3 Top association results in non-Hispanic whites

<table>
<thead>
<tr>
<th>RSID</th>
<th>Chr</th>
<th>Position</th>
<th>Beta</th>
<th>SE</th>
<th>P-value</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10011796</td>
<td>4</td>
<td>89090877</td>
<td>0.2799</td>
<td>0.04959</td>
<td>1.99E-08</td>
<td>ABCG2</td>
</tr>
<tr>
<td>rs17211056</td>
<td>18</td>
<td>73183461</td>
<td>0.6488</td>
<td>0.1274</td>
<td>4.02E-07</td>
<td>SMIM21</td>
</tr>
<tr>
<td>rs3114020</td>
<td>4</td>
<td>89083666</td>
<td>0.2433</td>
<td>0.0504</td>
<td>1.54E-06</td>
<td>ABCG2</td>
</tr>
<tr>
<td>rs11152031</td>
<td>18</td>
<td>52533038</td>
<td>−0.2519</td>
<td>0.0522</td>
<td>4.93E-06</td>
<td>MYH11, NDE1</td>
</tr>
<tr>
<td>rs3762427</td>
<td>1</td>
<td>151867560</td>
<td>0.4158</td>
<td>0.08677</td>
<td>1.82E-06</td>
<td>ABCG2, PKD2</td>
</tr>
<tr>
<td>rs2231142</td>
<td>4</td>
<td>89052323</td>
<td>0.323</td>
<td>0.06748</td>
<td>1.86E-06</td>
<td>ABCG2</td>
</tr>
<tr>
<td>rs2199936</td>
<td>4</td>
<td>89264355</td>
<td>0.3038</td>
<td>0.06551</td>
<td>3.84E-06</td>
<td>ABCG2, PKD2</td>
</tr>
<tr>
<td>rs12922040</td>
<td>16</td>
<td>15869466</td>
<td>0.2415</td>
<td>0.05267</td>
<td>4.93E-06</td>
<td>MYH11, NDE1</td>
</tr>
</tbody>
</table>

All SNPs associated with uric acid change in response to allopurinol with \( P < 5 \times 10^{-5} \) in a cohort of 1,492 patients of European ancestry. Annotated genes are any gene found within 50 kb of the SNP. Chr, Chromosome. Beta, Beta-coefficient. A positive beta coefficient means that the minor allele is associated with smaller change in SUA from baseline and hence poorer response, whereas a negative beta-coefficient means that the effect allele is associated with larger change in SUA from baseline and hence better response. SE, standard error.
hypersensitivity reactions including Stevens-Johnson syndrome and toxic epidermal necrolysis,\textsuperscript{8,9,21} as well as the results from the current study, it may be possible to use genetic biomarkers to identify patients who should not be on allopurinol therapy. These patients may represent a smaller fraction of gout patients who would benefit from more costly drug therapies.

**METHODS**

**Study population**

Participants were drawn from the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort, a subsample of the Kaiser Permanente Research Program on Genes, Environment, and Health (RPGEH). A detailed description of the cohort and study design can be found in dbGaP.\textsuperscript{22} The GERA cohort comprises a sample of 110,266

Figure 3  \(\text{BCRP-mediated transport of allopurinol, oxypurinol, and pitavastatin. Cells stably transfected with empty vector or reference ABCG2 were incubated with (a) [3H]-allopurinol or (b) [3H]-oxypurinol with or without Ko-143, a selective BCRP inhibitor. BCRP is an efflux transporter, so BCRP transport would lead to a lower accumulation of allopurinol in the HEK293 cells. (c) rs2231142 affects BCRP transport of allopurinol and oxypurinol. Cells stably transfected with reference ABCG2 or the ABCG2 Q141K variant were incubated with [3H]-allopurinol or [3H]-oxypurinol. (d) Allopurinol and oxypurinol did not affect BCRP transport of pitavastatin. Cells stably transfected with reference ABCG2 were incubated with [3H]-pitavastatin with or without Ko143, allopurinol (0.5, 1, and 2 mM), or oxypurinol (0.5, 1, and 2 mM). Using Dunnett’s multiple comparison test, only cells incubated with Ko-143 (10 \text{\textmu}M) was statistically significant from cells incubated with vehicle (\(P < 0.0001\)). Values +/- standard errors are shown in these figures.\)
adult members of Kaiser Permanente Medical Care Plan, Northern California Region (KPNC) with high-density single nucleotide polymorphism (SNP) markers linked to comprehensive electronic health records (EHR) containing information on pharmacy utilization, laboratory test results, clinical diagnoses, and other clinical utilization. All patients signed broad consent forms for use of their data in research on health and disease. In addition, the Kaiser Permanente Northern California Division of Research's Institutional Review Board (IRB) reviewed and approved the current study. The average age of the multiethnic GERA cohort was about 63 years at the time of sample collection and includes about 81% self-identified NHW participants, and 19% self-identified ethnic minorities (7% Asian, 3.5% African American, 7% Latinos, and 1.5% Other). The EHRs were examined to identify all subjects with an allopurinol prescription who also had SUA measurements. Allopurinol prescriptions within 0.2 years of each other were combined into a single prescription period while still tracking any changes in prescribed dosage. SUA measurements were classified as being untreated (not taken during or within 0.5 years of the end of an allopurinol prescription), treated (taken during or within 0.3 years of the end of an allopurinol prescription), or neither. In addition, the EHR was queried for body mass index (BMI), gender, age at time of the SUA measurements, allopurinol dose, and UA-affecting concomitant medications, defined as diuretics and urate-lowering drugs.

Participants for this study were selected as individuals having an allopurinol prescription with an untreated SUA obtained at most 1.5 years prior to the prescription, and a treated value for the same prescription. If multiple prescriptions fulfilled the criteria, the earliest prescription was used to try to lessen any possible interactions with worsening health conditions or development of drug tolerance.

Genotyping

Genome-wide genotypes on GERA cohort members were generated at the University of California, San Francisco, and underwent detailed quality control. Saliva samples were obtained from the participants and normalized to 10 ng/μl at Kaiser Permanente. Genotyping was conducted using one of four race/ethnic-specific Affymetrix Axiom arrays (Santa Clara, CA). These were the Axiom_KP_UCSF_EUR, _EAS, _AFR, and the _LAT array and were designed to maximize coverage across the NHW, East Asians, African Americans, and Latinos.23,24 The resulting calls were evaluated and samples with DashQC values <0.82 or with sample call rates <97% were removed, leaving 103,006 well-genotyped samples. Additional QC steps included filtering of autosomal SNPs with large allele frequency differences between genders or SNPs with poor overall call rates among other criteria. Full details can be found in the GERA dbGaP submission.22 In total, 670,176 SNPs, 801,830 SNPs, 877,845 SNPs, and 708,134 SNPs in the NHW, Latinos, African Americans, and East Asians, respectively, passed initial QC. For this study, additional filters removed polymorphisms with more than 10% missing genotypes or less than 1% minor allele frequency.

Genetic ancestry

EIGENSOFT4.25 was used to compute principal components (PC) separately for individuals on the four different arrays using 144,799 high-quality SNPs common to all arrays. Since the calculations were computationally intensive in NHW, they were instead run on a subset of individuals (N = 20,000) with the remaining individuals projected into the same space. The results were validated in three random sets of NHW from the cohort. Full details can be found in the GERA dbGaP submission.22 These PCs were used in the GWAS to adjust for genetic ancestry.

Imputation

We performed imputation of subject genotypes separately by array. The genotypes in our cohort were first prephased with Shape-it v2.r727,28 Subsequently, we imputed variants from the 1000 Genomes Project29 as a cosmopolitan reference panel with Impute2 v2.3.0.28–30 We used the quality control r² metric from Impute2, which estimates the correlation of the true genotype to the imputed genotype,31 and set a cutoff filter to eliminate SNPs with an r² < 0.3.

Association

Association analysis was conducted using a linear regression model in PLINK v1.07.32 Initial analysis focused on NHW subjects, as they formed the largest homogenous subgroup. To calculate the change in SUA (our measure of pharmacoresponse), we selected the untreated value as the baseline measurement; if multiple values were available, we chose the measurement closest to treatment initiation. We then adjusted the baseline measurements for BMI, age, gender, and concomitant medications by linear regression. The same was done for the treated values found in our cohort. We then calculated the difference between the treated SUA residuals and the residuals for the associated baseline value. A strong nonlinear relationship between cumulative dose and SUA difference was observed, so we adjusted for cumulative dose using a spline regression. If multiple treated values were present for a particular patient, we used the median change after the cumulative dose adjustment step for subsequent analyses. In the PLINK linear regression model, covariates were included for baseline SUA, age, current dose, BMI, gender, concomitant medications, and the top population structure principal component, as further principal components were much weaker and not significantly associated with the outcome. As a QA step, top associated SNPs were checked for extreme HWE departures in our samples.

The imputed data were analyzed using a meta-analysis. Association analysis was conducted separately on subjects in each race/ethnicity group (NHW, East Asian, Latino, and African American) using all typed and imputed SNPs and using the same covariates as the original analysis with the appropriate principal components for that ethnic group. The results were then combined in a transethnic meta-analysis in PLINK using a random effects model. As described elsewhere,22 a small subset of NHW were genotyped on arrays other than the NHW array. They were excluded from the initial GWAS of NHW but included in the meta-analysis using imputed genotypes.

Cell lines and cell culture

Human embryonic kidney (HEK) 293 cells expressing vector (pcDNA3) or vector expressing myc-tagged ABCG2 construct were provided by Dr. Jian-Ting Zhang (Indiana University, Indianapolis, IN).34,35 The cells were maintained in DME-H21, supplemented with 10% FBS, 2 mM glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, and 200 µg/mL G418 at 37°C in 5% CO2. HEK293 cells expressing vector only (pcDNA3.1) and cells expressing ABCG2 or ABCG2-Q141K were provided by Dr. Susan E. Bates (National Institutes of Health, Bethesda, MD).36 The cells were maintained in DME-H21 as described above and in 2 mg/mL G418.

Cytotoxicity assay

HEK293 cells stably expressing vector only or ABCG2 or ABCG2-Q141K provided by Dr. J.T. Zhang and Dr. S.E. Bates were seeded at 6,000 cells
per well in 96-well poly-d-lysine-coated plates (BD Biosciences, San Jose, CA). On the following day, cells were exposed to growth medium containing mitoxantrone (200 nM, 100 nM, 50 nM, 25 nM, 12.5 nM) or vehicle for 72 hours. Cell density was measured using the CellTiter-Glo cell viability kit (Promega, Madison, WI) according to the manufacturer’s instructions. Proliferation of each HEK293 cell line after 72 hours was normalized to the density measured on cells treated with mitoxantrone to vehicle treatment. The concentration of mitoxantrone to inhibit 50% of the cell proliferation (IC\textsubscript{50}) was computed by fitting the data using GraphPad Prism (v. 5.0, GraphPad Software, San Diego, CA).

**Cell accumulation studies**

HEK293 cells expressing vector only or ABCG2 or ABCG2-Q141K cDNA constructs were seeded at 2.5 × 10\textsuperscript{5} cells per well in 24-well poly-d-lysine-coated plates (BD Biosciences). Mixtures containing the following were prepared for cell accumulation studies: HBSS with trace amount of radiolabeled compound ([\textsuperscript{3}H]-allopurinol or [\textsuperscript{3}H]-oxypurinol) and its unlabeled compound, allopurinol (1 \textmu M) or oxypurinol (1 \textmu M), were mixed with ABCG2 inhibitor, Ko-143 (10 \textmu M), or with vehicle (0.2\% DMSO). After the cells were washed twice and incubated with HBSS for 15 minutes, HBSS were removed and cells were incubated with the mixtures for 30 minutes at 37\°C. The studies associated with determining accumulation of [\textsuperscript{3}H]-pitavastatin in BCRP-expressing cells, allopurinol (0.5, 1 and 2\%\%) or oxypurinol (0.5, 1 and 2\%\%) or Ko-143 (10 \textmu M as positive control) or vehicle (1.0 \textmu M DMSO) were used. The accumulation studies were terminated by washing cells twice with 1.0 \textmu L ice-cold HBSS, followed by addition of 800 \mu L of lysis buffer (0.1% SDS v/v, 0.1 \textmu M NaOH). Intracellular radioactivity was determined by scintillation counting and normalized per well of protein content as measured by bicinchoninic acid protein assay (Pierce, Rockford, IL). The method for this study was modified from Abla et al.\textsuperscript{3} Data were analyzed by two-tailed unpaired t-test. P-values <0.05 were considered statistically significant.

Additional supporting information may be found in the online version of this article.

**ACKNOWLEDGMENTS**

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**CONFLICT OF INTEREST**

Dr Giacomini reports personal fees from Apricity Therapeutics, grants from Pfizer, grants from Sanofi Aventis, grants from Astra Zeneca, and grants from GSK outside the submitted work. Dr Yee reports personal fees from Apricity Therapeutics, outside the submitted work.

**AUTHOR CONTRIBUTIONS**

CCW, CS, NR, and KMG designed the research. SWY, and XL performed the research. CCW, NR, KMG, TJH, MNK, YB, and LJ analyzed the data. CCW, KMG, and NR wrote the article. All authors reviewed and edited the final report.

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