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ARTICLE

An association study of *ABCG2* rs2231142 on the concentrations of allopurinol and its metabolites

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Abstract

ABCG2 is a gene that codes for the human breast cancer resistance protein (BCRP). It is established that rs2231142 G>T, a single nucleotide polymorphism of the *ABCG2* gene, is associated with gout and poor response to allopurinol, a uric acid-lowering agent used to treat this condition. It has also been suggested that oxypurinol, the primary active metabolite of allopurinol, is a substrate of the BCRP. We thus hypothesized that carrying the rs2231142 variant would be associated with decreased oxypurinol concentrations, which would explain the lower reduction in uric acid. We performed a cross-sectional study to investigate the association between the *ABCG2* rs2231142 variant and oxypurinol, allopurinol, and allopurinol riboside concentrations in 459 participants from the Montreal Heart Institute Hospital Cohort. Age, sex, weight, use of diuretics, and estimated glomerular filtration rate were all significantly associated with oxypurinol plasma concentration. No association was found between rs2231142 and oxypurinol, allopurinol and allopurinol riboside plasma concentrations. Rs2231142 was not significantly associated with daily allopurinol dose in the overall population, but an association was observed in men, with T carriers receiving higher doses. Our results do not support a major role of *ABCG2* in the pharmacokinetics of allopurinol or its metabolites. The underlying mechanism of the association between rs2231142 and allopurinol efficacy requires further investigation.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

ABCG2 is a gene that codes for the human BCRP. It is established that rs2231142 G>T, a single nucleotide polymorphism of the *ABCG2* gene, is associated with gout and poor response to allopurinol, a uric acid-lowering agent used to treat this condition. It is not clear if pharmacokinetic changes are involved in the underlying mechanism of those observations.

WHAT QUESTION DID THIS STUDY ADDRESS?
This study addressed whether the rs2231142 loss-of-function variant is associated with decreased oxypurinol concentrations.
WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
Our results do not support a major role of *ABCG2* in the pharmacokinetics of allopurinol or its metabolites.
HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
Further investigations of the links between *ABCG2* rs2231142 and the pharmacodynamics of allopurinol are needed.

INTRODUCTION

The association of the human breast cancer resistance protein (BCRP) gene, *ABCG2*, on the pharmacokinetics of multiple drugs has been shown.[1] Its effect is related to its capacity to modulate the transport of drugs in multiple organs, including the gut, the liver, as well as the kidneys.[2] An increased gene expression leads to a reduction in the absorption of its substrates and an increase in their elimination in the kidneys and the biliary tract.[2–4] Knockout models have shown that its nonexpression leads to increased circulating concentrations of its substrates.[5] Concordantly, in humans, accumulating data show that carriers of the reduced-function missense variation at rs2231142 in the *ABCG2* protein, also described as p.Gln141Lys (Q141K), present higher concentrations of such drugs.[1]

Individuals possessing the minor allele (T, corresponding to 141 K) at rs2231142 present higher uric acid levels and increased risk of gout.[6] Moreover, existing data suggests that this variant could modulate the uric acid-lowering response to allopurinol,[7–9] a drug mainly prescribed for long-term prevention of recurrent episodes of gout.[10–13] Allopurinol, a structural analogue of hypoxanthine, is metabolized in the liver to the corresponding xanthine analogue, oxypurinol. This active metabolite, which has a much longer half-life (~24 vs. ~1.5 h), is responsible for the majority of the uric acid-lowering effect of allopurinol.[10–13] Reduction in both the serum and urinary uric acid levels is caused by their inhibition of xanthine oxidase, the enzyme responsible for the conversion of hypoxanthine to xanthine and xanthine to uric acid.[14,15]

Allopurinol had been proposed to be a BCRP substrate,[8] but more recent data has suggested otherwise.[16] On the other hand, one study using isolated cells has shown that oxypurinol is a BCRP substrate.[8] Surprisingly, carriers of the 141K variant, who are expected to present higher concentrations of allopurinol/oxypurinol, were reported to present a decreased uric acid-lowering response to allopurinol.[7–9]

Thus, the mechanisms underlying the association between rs2231142 and response to allopurinol remain to be elucidated.[17] The aim of this study was thus to explore the association of *ABCG2* rs2231142 with concentrations of oxypurinol, allopurinol, and allopurinol riboside in the Montreal Heart Institute (MHI) hospital Cohort.

PATIENTS AND METHODS

Study design

We performed a cross-sectional study that included participants from the MHI Hospital Cohort taking allopurinol. The methods of MHI Hospital Cohort have been described previously.[18–21] Succinctly, the MHI Hospital Cohort is composed of individuals who have previously used the hospital's services and have provided informed consent to participate in the Biobank of the MHI Hospital Cohort. The Biobank of the MHI Hospital Cohort contains encoded data and biological material. These data provide information on the medical, genealogical, psychological, biological, pharmacological, and genetic profile of the participants. Samples (DNA and plasma) and clinical data used as part of this study were collected as previously described.[18–22] In the current analysis, for 24 patients, plasma was collected during follow-up, at which time a complete pharmacological and medical history was again completed and used as part of these analyses. All information was collected directly from participants, their medical records, and MHI electronic databases, with the exception of allopurinol, oxypurinol, and allopurinol d-ribose concentrations, which were measured from plasma as detailed below.

Study population

We included in this study self-reported "White" male and female patients aged ≥18 years with allopurinol being

a part of their routine pharmacotherapy at the time of plasma sampling. We included only participants who self-identified as "White," which represents 98% of the Biobank population, in order to minimize the risk of confounding due to population stratification. We excluded patients with a history of liver, kidney, or heart transplant because the genotype of the transplanted organ could differ from the genotype of the recipient.[23] No other specific illnesses related to allopurinol therapy were included or excluded from the cohort.

Study end points

The primary objective of this study was to explore the association of the *ABCG2* rs2231142 variant and concentrations of oxypurinol. The secondary objectives were to explore its association with concentrations of allopurinol and allopurinol riboside, as well as allopurinol daily dosing.

Measurement of oxypurinol, allopurinol, and allopurinol riboside concentrations

The quantification of all samples was performed at the Platform of Biopharmacy, Université de Montréal, in a blinded manner. The plasma samples were thawed on ice, vortex-mixed, and 50 µl were extracted by protein precipitation with the addition of 125 µl of the internal standard solution (7-methyl-xanthide at 1 µg/ml in 80% acetonitrile, 19% methanol, and 1% formic acid). Samples were centrifuged and 50 µl of supernatant was diluted with two volumes of water containing 0.1% formic acid. Seventeen calibration standards including allopurinol, oxypurinol, and allopurinol riboside ranging from 10 to 50,000 ng/ml were prepared in blank human plasma and were extracted as mentioned above. Samples were analyzed in the selective multiple reaction monitoring (MRM) mode using high pressure liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-MS/MS) in positive ion mode. Calibration curves were plotted using peak area ratios (analyte/internal standard) versus nominal analyte concentration, using a weighted 1/× quadratic regression resulting in lower limits of quantification (LLOQ) of 10 ng/ml for allopurinol and its metabolites oxypurinol and allopurinol riboside. As part of the statistical analyses, concentrations below the LLOQ were attributed a value of zero. More details regarding the quantification method and its validation are provided in the Supplementary Methods.

Genotyping

Participant's DNA was genotyped using a custom pharmacogenomic panel designed and developed at the Université de Montréal Beaulieu-Saucier Pharmacogenomics Centre (PGx Center) using the Agena Bioscience iPLEX Gold chemistry (Agena Bioscience, San Diego, CA). Briefly, the target regions of genomic DNA were amplified by polymerase chain reaction (PCR) using primers designed at the PGx Center. Unincorporated dNTPs were then neutralized using SAP reaction. Single base extension was conducted using iPLEX Gold extension chemistry (Agena Bioscience) and extension probes designed at the PGx Center producing allele-specific extension products of different masses. Extension reactions were dispensed on a SpectroChipII Array using the MassARRAY Nanodispenser, data was acquired using the MassARRAY Analyzer Compact matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer and the different extended mass intensities were analyzed using the MassArray Typer Analyzer application version 4.0.26.75 of Typer software version 4. Genotyping was performed on the DNA samples of the 459 eligible participants along with controls, including samples NA20769, NA19764, NA21105, and NA12812 from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research.

Statistical analysis

We conducted descriptive statistics of demographic and clinical characteristics for all included participants. Means and SDs were reported for quantitative variables, whereas for categorical variables, counts and percentages were presented. Linear regression models were used to test the association between the rs2231142 variant and the study end points. Additional analyses were conducted with models using the "concentrations/allopurinol daily dose" ratio for oxypurinol, allopurinol, and allopurinol ribose concentrations. Moreover, in order to validate the findings from multivariable regressions, forward inclusion and backward exclusion criteria analyses were conducted using $p < 0.15$ for entry and $p > 0.05$ for exit criteria. Candidate adjustment variables were considered iteratively, and included age, sex, allopurinol dose, weight, use of diuretic, and estimated glomerular filtration rate (eGFR). Outcome measurements were log-transformed to satisfy the normality assumption, and the resulting distributions of residuals from models with transformed outcomes were more normal than those resulting from

the models with untransformed values. Given the long elimination half-life of oxypurinol, patients ($n = 10$) with nonquantifiable concentration were considered to not have taken allopurinol for an extended period of time and were excluded from multivariable analyses. In models of allopurinol and allopurinol riboside concentrations, these 10 patients were also excluded and concentrations below the LLOQ for these two compounds were attributed a value of zero in the analyses. Serum creatinine was not available in 50 patients, which were excluded from models adjusted for eGFR. Two-tailed tests were used and p values below 0.05 were considered statistically significant. All analyses were carried using SAS version 9.4 (SAS Institute, Cary, NC).

Ethics statement

This study was conducted according to the Management Policy of the MHI Hospital Cohort and was approved by the Management Committee of the Biobank as well as the Scientific and Ethics committees of the MHI. All patients agreed to participate in the Hospital Cohort and signed a consent form.

RESULTS

Study population

A total of 459 participants were included. Patients were mainly men (85.8%), with a mean age of 69.4 ± 8.0 years. A majority of patients presented cardiovascular risk factors, including hypertension (86.5%), type 2 diabetes (41.0%), dyslipidemias (86.9%), or cardiovascular history, including myocardial infarction (39.9%). Expectedly, patients were treated with a wide variety of cardiovascular medications (Table 1).

Allopurinol dosing and allopurinol and its metabolites concentrations

Total daily doses of allopurinol ranged from 42.9 mg (100 mg 3 days per week) to 600 mg. The mean daily dose of allopurinol overall was 194.1 ± 77.3 mg. In the overall population, the mean ± SD concentrations of oxypurinol, allopurinol, and allopurinol riboside were 13025.9 ± 8718.0, 269.0 ± 354.6, and 221.5 ± 205.9 ng/ml, respectively. Of the 459 patients, 449 (97.8%) had quantifiable concentration of oxypurinol, whereas 344 (75.0%) and 425 (92.6%) had quantifiable concentrations of allopurinol and allopurinol riboside, respectively.

TABLE 1 Baseline characteristics

Characteristics	All *N* = 459
Age	69.42 ± 7.98
Sex, *n* (%)	
Male	394 (85.84%)
Female	65 (14.16%)
Smoking status, *n* (%)	
Past-smoker	316 (68.85%)
Current-smoker	21 (4.58%)
Weight (kg), mean (SD)	90.32 ± 18.38
BMI, mean (SD)	31.50 ± 5.53
Hypertension, *n* (%)	397 (86.49%)
Diabetes, *n* (%)	
Type 1	1 (0.22%)
Type 2	188 (40.96%)
Dyslipidemia, *n* (%)	399 (86.93%)
Myocardial infarction, *n* (%)	183 (39.87%)
Chronic renal failure	122 (26.58%)
Medications	
Aspirin, *n* (%)	322 (70.15%)
Other antiplatelet agents, *n* (%)	70 (15.25%)
ACE inhibitors, *n* (%)	168 (36.60%)
Angiotensin II receptor blockers, *n* (%)	177 (38.56%)
Beta-blocker, *n* (%)	326 (71.02%)
Calcium channel blocker, *n* (%)	160 (34.86%)
Warfarin, *n* (%)	119 (25.93%)
Novel oral anticoagulants, *n* (%)	19 (4.14%)
Digoxin, *n* (%)	55 (11.98%)
Amiodarone, *n* (%)	20 (4.36%)
Diuretics, *n* (%)	275 (59.91%)
Statins, *n* (%)	371 (80.83%)
Fibrates, *n* (%)	16 (3.49%)
Other hypolipidemic agents, *n* (%)	55 (11.98%)
Oral hypoglycemic agents, *n* (%)	163 (35.51%)
Insulin, *n* (%)	36 (7.84%)
Serum creatinine and eGFR ($n = 409$)	
Serum creatinine, mmol/L, mean (SD)	119.30 ± 57.37
eGFR[a], ml/min/1.73 m^2, mean (SD)	59.77 ± 21.02
Genetic variables, *n* (%)	
rs2231142	
G	786 (85.62%)
T	132 (14.38%)
G/G	337 (73.42%)
G/T	112 (24.40%)
T/T	10 (2.18%)

Note: The p value for continuous variables: Kruskal–Wallis test, categorical variables: Chi-Square test or Fisher exact test.

Abbreviations: ACE, angiotensin-converting enzyme; BMI, body mass index; eGFR, estimated glomerular filtration rate.

[a]eGFR (according to the CKD-EPI equation).

Oxypurinol

No significant association was seen between rs2231142 (Table 2, Figure 1a) and oxypurinol concentrations in unadjusted and adjusted models. In contrast, age, sex, allopurinol daily dose, weight, diuretic use, and eGFR were all associated with oxypurinol concentrations, although the effect of sex was attenuated in the model that included eGFR. Globally, these variables explained 46.7–53.6% of interindividual's concentrations of oxypurinol. Allopurinol dose was the factor which explained the greatest proportion of the variance in oxypurinol concentrations, with 24.8–26.4%. Age and eGFR were the two other major determinants. An additional model using oxypurinol concentrations/allopurinol dose ratio instead of oxypurinol concentrations produced similar results (Figure 1d, Table S1).

In the sex-stratified analyses (Tables S2 and S3), no significant association was observed between oxypurinol concentrations and rs2231142, although nonsignificant trends were observed in men in some models (all $p > 0.08$, see Supplementary Methods).

Allopurinol

We did not observe any significant association between rs2231142 and allopurinol concentrations (Table 3, Figure 1b). Allopurinol dose and weight were consistently associated with allopurinol concentrations in multivariable analyses (all $p < 5.0 \times 10^{-12}$ and $p < 5.0 \times 10^{-4}$, respectively). Additionally, the model assessing allopurinol concentrations/allopurinol dose ratio also showed a consistent association with weight (Figure 1e, Table S4).

Sex-stratified analyses revealed no significant associations between allopurinol concentrations and rs2231142 (Tables S5 and S6).

Allopurinol riboside

We did not observe any significant association between rs2231142 and allopurinol riboside concentrations (Table 4, Figure 1c). Results of multivariable regression analyses showed that age, allopurinol dose, and eGFR were significantly associated with allopurinol riboside concentrations (all $p < 0.05$; Table 4), with a more modest association with weight when adjusting for renal function. In the concentration/dose ratio model, trends were similar, apart from the effect of age that was no longer statistically significantly associated with allopurinol ribose concentrations/allopurinol dose ratio,

TABLE 2 Association between oxypurinol concentration and rs2231142

	Model 1		Model 2		Model 3		Model 4		R^2 Model 4	Model 5		R^2 Model 5
Effect	**Estimate (SE)**	***p* Value**	**Estimate (SE)**	***p* Value**	**Estimate (SE)**	***p* Value**	**Estimate (SE)**	***p* Value**	**46.68**	**Estimate (SE)**	***p* Value**	**53.59**
rs2231142	−0.015 (0.070)	0.8303	0.004 (0.066)	0.9541	−0.056 (0.056)	0.3149	−0.063 (0.052)	0.2240	0.01	−0.069 (0.053)	0.1889	0.03
Age	–	–	0.027 (0.004)	$\mathbf{3.1 \times 10^{-10}}$	0.034 (0.004)	$\mathbf{1.7 \times 10^{-19}}$	0.024 (0.003)	$\mathbf{8.1 \times 10^{-12}}$	8.71	0.011 (0.004)	**0.0052**	9.66
Sex	–	–	−0.267 (0.095)	**0.0050**	−0.381 (0.080)	$\mathbf{2.8 \times 10^{-6}}$	−0.188 (0.077)	**0.0147**	1.61	−0.121 (0.078)	0.1224	1.84
Allopurinol dose	–	–	–	–	0.005 (0.0004)	$\mathbf{2.4 \times 10^{-35}}$	0.005 (0.0003)	$\mathbf{2.2 \times 10^{-41}}$	26.35	0.006 (0.0004)	$\mathbf{2.3 \times 10^{-43}}$	24.84
Weight	–	–	–	–	–	–	−0.011 (0.002)	$\mathbf{7.4 \times 10^{-12}}$	4.10	−0.010 (0.002)	$\mathbf{2.6 \times 10^{-10}}$	4.36
Use of diuretic	–	–	–	–	–	–	0.384 (0.055)	$\mathbf{9.8 \times 10^{-12}}$	5.92	0.285 (0.058)	$\mathbf{1.1 \times 10^{-6}}$	2.91
eGFR	–	–	–	–	–	–	–	–	–	−0.012 (0.002)	$\mathbf{2.0 \times 10^{-13}}$	9.94

Note: Model 1: crude model; model 2: model adjusted for age and sex; model 3: model adjusted for age, sex, and allopurinol dose; model 4: model adjusted for age, sex, allopurinol dose, weight, and use of diuretic; and model 5: model adjusted for age, sex, allopurinol dose, weight, use of diuretic, and eGFR. Intercepts for model 1: 9.288, model 2: 7.648, model 3: 6.321, model 4: 7.520, and model 5: 8.998.

Significant *p* values (< 0.05) are highlighted in bold.

Abbreviation: eGFR, estimated glomerular filtration rate.

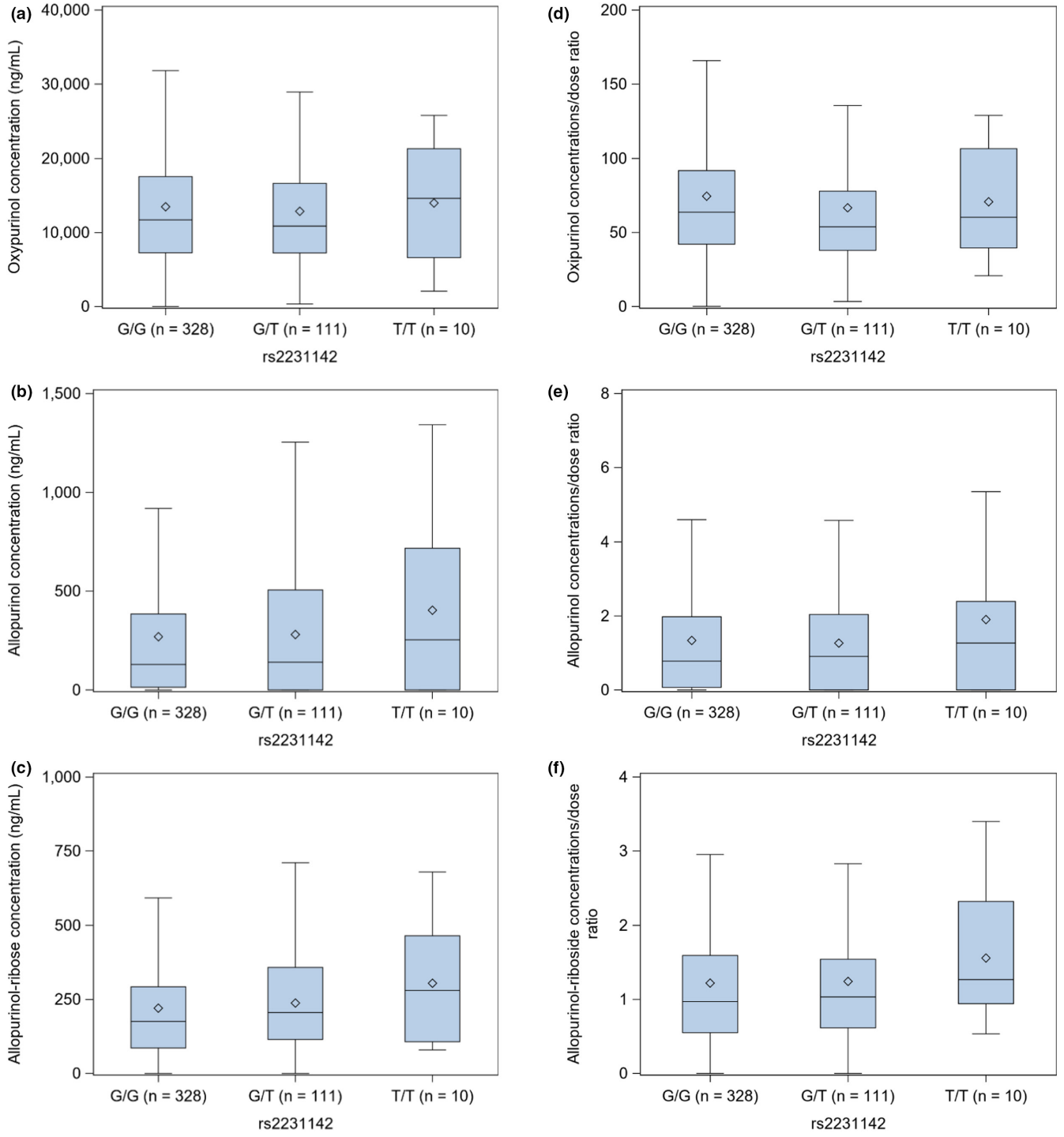

FIGURE 1 Concentrations and concentrations/dose ratio of allopurinol, oxypurinol, and allopurinol riboside. (a) Concentration of oxypurinol by rs2231142 genotypes. (b) Concentration of allopurinol by rs2231142 genotypes. (c) Concentration of allopurinol riboside by rs2231142 genotypes. (d) Concentration/dose ratio of oxypurinol by rs2231142 genotypes. (e) Concentration/dose ratio of allopurinol by rs2231142 genotypes. (f) Concentration/dose ratio of allopurinol riboside by rs2231142 genotypes.

whereas weight reached significance level (Figure 1f, Table S7).

Sex-stratified analyses revealed no significant association related to rs2231142 and allopurinol ribose concentrations (Tables S8 and S9).

Allopurinol daily dose

In the overall population, we did not find any significant association between rs2231142 and daily allopurinol dose, although trends were observed throughout the models

TABLE 3 Association between allopurinol concentration and rs2231142

	Model 1		Model 2		Model 3		Model 4		R^2 Model 4	Model 5		R^2 Model 5
Effect	**Estimate (SE)**	***p* Value**	**Estimate (SE)**	***p* Value**	**Estimate (SE)**	***p* Value**	**Estimate (SE)**	***p* Value**	**15.69**	**Estimate (SE)**	***p* Value**	**15.66**
rs2231142	0.038 (0.234)	0.8716	0.035 (0.233)	0.8820	−0.087 (0.222)	0.6958	−0.160 (0.218)	0.4652	0.01	−0.217 (0.232)	0.3521	0.01
Age	–	–	0.028 (0.015)	0.0575	0.042 (0.014)	**0.0031**	0.025 (0.015)	0.0856	0.72	0.015 (0.017)	0.3838	1.14
Sex	–	–	0.683 (0.334)	**0.0418**	0.442 (0.319)	0.1666	0.655 (0.325)	**0.0446**	0.91	0.847 (0.346)	**0.0147**	1.24
Allopurinol dose	–	–	–	–	0.011 (0.002)	$\mathbf{4.4 \times 10^{-12}}$	0.011 (0.002)	$\mathbf{3.4 \times 10^{-14}}$	10.10	0.011 (0.002)	$\mathbf{1.6 \times 10^{-12}}$	9.41
Weight	–	–	–	–	–	–	−0.028 (0.006)	$\mathbf{1.4 \times 10^{-5}}$	3.93	−0.025 (0.007)	**0.0003**	3.15
Use of diuretic	–	–	–	–	–	–	−0.070 (0.232)	0.7644	0.02	−0.128 (0.254)	0.6148	0.05
eGFR	–	–	–	–	–	–	–	–	–	−0.012 (0.007)	0.0718	0.65

Note: Model 1: crude model; model 2: model adjusted for age and sex; model 3: model adjusted for age, sex, and allopurinol dose; model 4: model adjusted for age, sex, allopurinol dose, weight, and use of diuretic; and model 5: model adjusted for age, sex, allopurinol dose, weight, use of diuretic, and eGFR. Intercepts for model 1: 4.039, model 2: 1.508, model 3: −1.256, model 4: 2.162, and model 5: 3.153.

Significant *p* values (< 0.05) are highlighted in bold.

Abbreviation: eGFR, estimated glomerular filtration rate.

TABLE 4 Association between allopurinol riboside concentration and rs2231142

	Model 1		Model 2		Model 3		Model 4		R^2 Model 4	Model 5		R^2 Model 5
Effect	**Estimate (SE)**	***p* Value**	**Estimate (SE)**	***p* Value**	**Estimate (SE)**	***p* Value**	**Estimate (SE)**	***p* Value**	**12.80**	**Estimate (SE)**	***p* Value**	**16.48**
rs2231142	0.122 (0.137)	0.3729	0.134 (0.135)	0.3204	0.070 (0.130)	0.5934	0.052 (0.130)	0.6871	0.18	0.032 (0.138)	0.8158	0.08
Age	–	–	0.036 (0.008)	$\mathbf{3.5 \times 10^{-5}}$	0.043 (0.008)	$\mathbf{3.7 \times 10^{-7}}$	0.036 (0.009)	$\mathbf{3.7 \times 10^{-5}}$	3.69	0.026 (0.010)	**0.0128**	4.67
Sex	–	–	0.193 (0.193)	0.3192	0.074 (0.187)	0.6906	0.181 (0.194)	0.3506	0.22	0.379 (0.205)	0.0659	0.49
Allopurinol dose	–	–	–	–	0.005 (0.001)	$\mathbf{1.8 \times 10^{-9}}$	0.006 (0.001)	$\mathbf{4.5 \times 10^{-10}}$	7.53	0.006 (0.001)	$\mathbf{1.2 \times 10^{-10}}$	7.94
Weight	–	–	–	–	–	–	−0.009 (0.004)	**0.0155**	1.07	−0.007 (0.004)	0.0898	0.68
Use of diuretic	–	–	–	–	–	–	0.110 (0.138)	0.4275	0.12	0.057 (0.151)	0.7040	0.03
eGFR	–	–	–	–	–	–	–	–	–	−0.013 (0.004)	**0.0011**	2.60

Note: Model 1: crude model; model 2: model adjusted for age and sex; model 3: model adjusted for age, sex, and allopurinol dose; model 4: model adjusted for age, sex, allopurinol dose, weight, and use of diuretic; and model 5: model adjusted for age, sex, allopurinol dose, weight, use of diuretic, and eGFR. Intercepts for model 1: 4.831, model 2: 2.194, model 3: 0.793, model 4: 1.888, and model 5: 2.903.

Significant *p* values (< 0.05) are highlighted in bold.

Abbreviation: eGFR, estimated glomerular filtration rate.

TABLE 5 Association between allopurinol daily dosing and rs2231142

Effect	Model 1		Model 2		Model 3		Model 4		R^2 Model 4	Model 5		R^2 Model 5
	Estimate (SE)	*p* Value	Estimate (SE)	*p* Value	Estimate (SE)	*p* Value	Estimate (SE)	*p* Value	6.13	Estimate (SE)	*p* Value	7.72
rs2231142	0.076 (0.041)	0.0648	0.069 (0.041)	0.0880	0.076 (0.040)	0.0589	0.078 (0.040)	0.0525	0.76	0.061 (0.043)	0.1533	0.43
Age	–	–	−0.007 (0.003)	**0.0061**	−0.005 (0.003)	0.0694	−0.005 (0.003)	**0.0430**	1.79	0.0004 (0.003)	0.8947	1.20
Sex	–	–	0.145 (0.058)	**0.0133**	0.114 (0.059)	0.0518	0.133 (0.060)	**0.0272**	1.34	0.099 (0.064)	0.1196	1.19
Weight	–	–	–	–	0.003 (0.001)	**0.0037**	0.003 (0.001)	**0.0100**	1.82	0.003 (0.001)	**0.0112**	2.33
Use of diuretic	–	–	–	–	–	–	0.061 (0.043)	0.1600	0.42	0.103 (0.047)	**0.0284**	0.56
eGFR	–	–	–	–	–	–	–	–	–	0.004 (0.001)	**0.0036**	2.01

Note: Model 1: crude model; model 2: model adjusted for age and sex; model 3: model adjusted for age, sex, and weight; model 4: model adjusted for age, sex, weight, and use of diuretic; and model 5: model adjusted for age, sex, weight, use of diuretic, and eGFR. Intercepts for model 1: 5.160, model 2: 5.528, model 3: 5.091, model 4: 5.111, and model 5: 4.494.
Significant *p* values (< 0.05) are highlighted in bold.
Abbreviation: eGFR, estimated glomerular filtration rate.

(p = 0.053 to 0.153; Table 5). The association between non-genetic variables and daily allopurinol dose was modest, with weight and eGFR showing the strongest associations. In our sex-stratified analyses (Tables S10 and S11), rs2231142 was associated with daily allopurinol dose in men (all $p < 0.05$), but not in women, although this association was attenuated in the model controlling for eGFR (p = 0.098), with the T allele associated with the use of higher allopurinol doses.

Sensitivity analyses

We repeated analyses on only patients with oxypurinol concentrations ≥3000 ng/ml, as performed by Wallace and Stamp.[7] This excluded 17 patients with concentrations below this threshold and, thus, a total of 432 patients were included in these analyses. Results were consistent with those in the overall population, with no significant associations between rs2231142 and oxypurinol, allopurinol, or allopurinol ribose concentrations (data not shown). We found no significant association between rs2231142 and allopurinol daily dose in this population, but a significant association was observed in men in the unadjusted (p = 0.018) and adjusted analyses (all $p < 0.03$, with the exception of the model also adjusting for eGFR).

Findings from the multivariable regressions were further validated with forward inclusion and backward exclusion criteria analyses. Results were generally similar and are shown in the Supplementary Material (Tables S12–S15). No association with rs2231142 was observed.

DISCUSSION

Our study provides supporting strong evidence for the importance of age, weight, diuretic use, eGFR, and to a lesser extent sex in determining oxypurinol concentrations, in addition to allopurinol daily dose. Collectively, these variables explained more than 50% of interindividual variability of oxypurinol concentrations. However, we did not find an association between the *ABCG2* genetic variant rs2231142 and concentrations of oxypurinol, the main active metabolite of allopurinol. There was also no significant association between rs2231142 and concentrations of allopurinol or allopurinol riboside. Similar results were obtained with concentration/dose ratio models. Consistent with the previous observation that T carriers have a blunted uric acid-lowering response to allopurinol,[7–9] we observed a significant association between rs2231142 and allopurinol dosing in men, with T carriers receiving higher doses.

ABCG2 encodes the efflux transporter BCRP, which is widely expressed in proximal tubules of the kidneys, the liver, and intestines.[24,25] *ABCG2* rs2231142 has been persistently reported to be associated with uric acid concentrations and gout,[6,7] with T carriers showing higher concentrations of uric acid and higher risk of gout. Although initial hypotheses underlined that transport and regulation of uric acid in the kidneys was central to this association, emerging data suggest that reduced uric acid excretion in the intestines may be even more important.[25] Furthermore, many studies have demonstrated significant rs2231142 by sex interactions for serum uric acid and gout risk,[26–28] with a greater effect in men than in women.[24,26] This suggests that BCRP and *ABCG2* may contribute to the higher uric acid levels and greater risk of gout in men compared to women.[24] The importance of sex in the modulation of this genetic association is also supported by data in a mouse model.[25]

T carriers have been consistently shown to demonstrate a poorer uric acid-lowering response to allopurinol in patients with gout.[7–9,17] Currently, it is uncertain whether this is related to higher baseline uric acid concentrations, an attenuated response to allopurinol (pharmacodynamics), or a modulation of allopurinol/oxypurinol pharmacokinetics, which would lead to reductions in oxypurinol concentrations. Regarding the latter, conflicting data exist regarding the impact of *ABCG2*/BCRP on allopurinol transport,[8,16] but in vitro data have shown that it transports oxypurinol.[16] Should lower concentrations of oxypurinol been observed, they would have been contrary to those made with multiple other BCRP substrates. For example, T carriers present high concentrations of rosuvastatin[1,29] and imatinib,[30] and, in many cases, greater therapeutic effect,[31,32] which is concordant with the reduction in the activity of this efflux transporter induced by this genetic variation.

In the current study, we saw no association between rs2231142 and oxypurinol, allopurinol, or allopurinol riboside plasma concentrations. Thus, our results do not support a major role of *ABCG2* in the pharmacokinetics of allopurinol or its metabolites. Although in vitro studies have provided supporting evidence for the implication of BCRP/*ABCG2* in oxypurinol transport, pharmacogenomic studies have provided conflicting evidence for the modulatory role of rs2231142 on oxypurinol concentrations. One study of 688 patients did not find any significant association between *ABCG2* Q141K (rs2231142) and oxypurinol concentration after considering allopurinol dose, eGFR, and the use of diuretics in a multivariable analysis.[17] However, oxypurinol levels were numerically lower in the T carrier group in that study. The same group then investigated factors that may influence the uric acid-lowering response to allopurinol and the relationship between allopurinol dose and change in oxypurinol concentration for each 100 mg increase in allopurinol dose in a randomized, controlled, parallel-group, comparative clinical trial of 129 patients.[33] The investigators reported that body mass index (BMI), creatinine clearance, *ABCG2* Q141K, and serum uric acid levels were all associated with allopurinol metabolism (change in oxypurinol concentration for each 100 mg increase in allopurinol dose). However, a significant association between allopurinol metabolism and *ABCG2* loss-of-function variant was only attained when considering exclusively homozygous groups of the Q141K allele (GG vs. TT), with TT carriers presenting lower change in oxypurinol concentrations for each 100 mg increment of allopurinol. Given the limited number of TT carriers ($n = 11$) in that study, the lack of association in the previous study,[17] and the fact that heterozygotes were found to have comparable oxypurinol concentrations to GG carriers in previous studies, we believe that the association, if any, between *ABCG2* rs2231142 and oxypurinol concentrations remains to be established. Given the emerging understanding that the effect of rs2231142 on the transport of uric acid and potentially other substrates in multiple organs may be influenced by sex and different disease states,[25] deciphering the underpinnings of the association between this variant and allopurinol response and pharmacokinetics could prove to be difficult in humans.

Nevertheless, because BCRP transports uric acid and the TT genotype at *ABCG2* rs2231142 is associated with higher uric acid levels, the reported reduced response or attainment of treatment targets[7,9] could also be the result of higher initial uric acid levels in variant carriers, or a more limited capacity to excrete uric acid upon treatment with allopurinol. Our observation that men carrying the T allele were treated with higher allopurinol doses is consistent with lower responsiveness to allopurinol in these individuals.[24,26]

Strengths and limitations

A strength of this study is that we have directly measured concentrations of oxypurinol, allopurinol, and allopurinol riboside. Another strength is that results were drawn from a polymedicated population presenting multiple comorbidities, which can more accurately capture the real clinical setting conditions. This has enabled the joint assessment of the different contributing factors in adjusted models, which explained ~50% of the interindividual variability in oxypurinol concentrations.

One limitation is that the cross-sectional nature of our study did not allow for the evaluation of changes

over time in uric acid concentrations. However, extensive data support the fact that T carriers at *ABCG2* rs2231142 have a decreased uric acid-lowering response to allopurinol. Moreover, because serum creatinine was not available in all patients, adjustment for eGFR was not possible in the overall study population. Furthermore, we have only assessed the genetic variant rs2231142, and different variants at the *ABCG2* gene may have captured the variability in the gene expression or gene product activity to different extents. Our population was enriched for individuals of European ancestry, and further investigations in populations of diverse ancestral origins may provide different results. Furthermore, we cannot exclude a modest impact of the variant. In our sample of patients with quantifiable oxypurinol concentrations, given our sample size of 449 participants, an allele frequency of 0.14 and a 95% level of confidence, the expected effect size, with a power of 80%, is 0.1985 (for logarithm of oxypurinol concentration). This is equivalent to a 21.96% change in oxypurinol concentrations per variant allele. Finally, considering the multiple models analyzed, the association between rs2231142 and allopurinol dose should be interpreted with caution.

CONCLUSION

Our results do not support a major role of *ABCG2* in the pharmacokinetics of allopurinol or its metabolites. Yet, the association between rs2231142 and higher allopurinol doses in men supports the implication of this variant in the response to allopurinol. Our study provides evidence that age, daily allopurinol dose, weight, diuretic use, eGFR, and, to a lesser extent, sex, are all important determinants of oxypurinol plasma concentrations or its ratio to the allopurinol dose.

AUTHOR CONTRIBUTIONS

M.-O.P., I.S.-J., M.J., E.O., M.-J.G., I.M., M.-P.D., and S.dD. wrote the manuscript. G.L., I.M., J.-L.R., J.-C.T., M.-P.D., and S.dD. designed the research. M.-O.P., G.L., I.S.-J., M.J., M.-J.G., I.M., D.B., J.-C.T., M.-P.D., and S.dD. performed the research. M.-O.P., E.O., I.M., M.-P.D., and S.dD. analyzed the data. G.L., I.S.-J., M.J., and M.-P.D. contributed new reagents/analytical tools.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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