The aims of this study were to determine factors that predict serum urate (SU) lowering response to allopurinol and the conversion of allopurinol to oxypurinol, and to determine a minimum therapeutic oxypurinol concentration. Data from 129 participants in a 24-month open, randomized, controlled, parallel-group, comparative clinical trial were analyzed. Allopurinol dose, SU, and plasma oxypurinol concentrations were available at multiple time points. The slope for the association between allopurinol dose and SU was calculated as a measure of sensitivity to allopurinol. The slope for the association between allopurinol dose and oxypurinol was calculated as a measure of allopurinol metabolism. Receiver operating characteristic (ROC) curves were used to identify a minimum oxypurinol concentration predictive of SU < 6 mg/dL. There was a wide range of SU concentrations for each allopurinol dose. The relationship between sensitivity to allopurinol and allopurinol metabolism for each 100 mg allopurinol dose increase varied between individuals. Body mass index ($P = 0.023$), creatinine clearance (CrCL; $P = 0.037$), $ABCG2$ Q141K ($P = 0.019$), and SU ($P = 0.004$) were associated with sensitivity to allopurinol. The minimum oxypurinol concentration for achieving the urate target was found to be about 104 $\mu$mol/L, but predictive accuracy was poor (ROC curve area under the curve (AUC) 0.65). The minimum therapeutic oxypurinol concentration was found to increase with decreasing renal function. Although there is a positive relationship between change in oxypurinol and change in SU concentration, a minimum therapeutic oxypurinol is dependent on CrCL and cannot reliably predict SU target. Other variables, including $ABCG2$ Q141K genotype, impact on sensitivity to allopurinol (ACTRN12611000845932).

Study Highlights

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

- There is large interindividual variability in the dose of allopurinol required to achieve a target serum urate (SU) concentration in the management of gout. Given this variability, monitoring plasma oxypurinol to guide allopurinol dosing may be an advantage.
- The study addresses two important questions: (i) what factors influence the urate-lowering response to allopurinol and relationship between allopurinol dose and oxypurinol concentration, and (ii) is there a minimum therapeutic oxypurinol concentration to achieve a target SU concentration?

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

- This study shows that body mass index, creatinine clearance, $ABCG2$ Q141K, and baseline urate all independently influence the allopurinol sensitivity and allopurinol metabolism. The minimum oxypurinol concentration for achieving the urate target was found to be about 104 $\mu$mol/L, but predictive accuracy was poor (receiver operating characteristic curve area under the curve (AUC) 0.65). The minimum therapeutic oxypurinol concentration was found to increase with decreasing renal function.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**

- The information from this study may be used to help develop an allopurinol dose prediction tool.
led many clinicians to limit the dose of allopurinol. This is particularly the case in the setting of renal impairment where oxypurinol (the active metabolite of allopurinol) may accumulate as it is primarily excreted by the kidneys.

Current rheumatology society guidelines recommend a target SU concentration of < 6 mg/dL for all people with gout and < 5 mg/dL for those with more severe disease.\(^1\)\(^2\) Lack of a standardized allopurinol dose, with marked interindividual variability in the dose required to achieve target urate also contributes to failure to reach target urate.\(^3\) A number of variables have been reported to influence the dose of allopurinol required to reach target urate, including pre-urate lowering SU concentration, renal function, weight, diuretic use, and the presence of the lysine (K) allele of ABCG2 Q141K, which encodes a urate efflux transporter in the kidneys, liver, and intestine.\(^5\)-\(^7\)

Given the variability in allopurinol dose required to reach target urate, the ability to measure plasma oxypurinol to guide allopurinol dosing may be an advantage. Previous studies examining the relationship between oxypurinol and SU concentrations have been conflicting, with some showing no relationship\(^8\) and some an inverse relationship.\(^9\),\(^10\) Therefore, defining a therapeutic range has been challenging.\(^10\)

The aims of this analysis were to determine the factors that influence the urate-lowering response and increase in plasma oxypurinol concentration in response to allopurinol dose escalation, and to determine if there is a minimum therapeutic oxypurinol concentration required to achieve target urate.

**METHODS**

**Study design**

A 24-month open, randomized, controlled, parallel-group, comparative clinical trial was undertaken (ACTRN12611000845932). Ethical approval was obtained from the Multi-Regional Ethics Committee, New Zealand. Written informed consent was obtained from each participant. Full methods have been reported previously.\(^11\),\(^12\) One hundred eighty-three people with gout were randomized to continue current dose allopurinol for the first 12 months and then enter the dose escalation (DE) phase in the second 12 months (control/DE) or to begin allopurinol dose escalation immediately (DE/DE). Allopurinol was increased monthly until the SU concentration was < 6 mg/dL. For those with creatinine clearance (CrCl; calculated using Cockcroft and Gault equation) ≤ 60 mL/minute, allopurinol was increased by 50 mg increments and for those with CrCl > 60 mL/minute by 100 mg increments. For the purpose of this analysis, sensitivity to allopurinol was defined as the degree of change in urate (mmol/L) for a 100 mg increase in allopurinol dose and allopurinol metabolism was defined as change in oxypurinol (μmol/L) for each 100 mg increase in allopurinol. Samples for SU, creatinine, and plasma oxypurinol were obtained at least every 3 months at any time during the 24-hour dosing interval. SU and creatinine were determined by Canterbury Health Laboratories.

**Measurement of plasma oxypurinol.** High-performance liquid chromatography was used to measure plasma oxypurinol, as previously described.\(^13\) In brief, 0.1 mL plasma was mixed with 0.1 mL of water, vortexed briefly, and 0.1 mL of 1 M perchloric acid added to precipitate the proteins. After centrifugation, 20 μL of supernatant was injected into the high-performance liquid chromatography system. Chromatographic separation was performed on a C8 column (Agilent Zorbax Eclipse XDB-C8 5 μm, 150 × 4.6 mm i.d.). The mobile phase was 0.01 M sodium phosphate buffer pH 3.5 containing 0.8% acetonitrile at a flow rate of 1.0 mL/minute. Eluting peaks were detected at 254 nm. Under these conditions, allopurinol and oxypurinol eluted at 3.9 and 4.8 minutes, respectively. Allopurinol and oxypurinol standard curves were linear (r² > 0.99) up to 294 mmol/L. Absolute recoveries from plasma were >90% for both allopurinol (at 1.2, 37, and 294 mmol/L) and oxypurinol (at 1.0, 33, and 263 mmol/L). Intra-day and inter-day coefficients of variation at the above concentrations were <9%, and the limit of quantification was 0.2 μmol/L for both allopurinol and oxypurinol. Participants were considered to be nonadherent with allopurinol if plasma oxypurinol was < 20 μmol/L and were excluded from the analysis.

**Genotyping.** Peripheral blood was collected (3–5 mL) and genomic DNA extracted using guanidine isothiocyanate. Genotyping was performed using the predesigned single nucleotide polymorphism TaqMan assays for ABCG2 single nucleotide polymorphisms rs2231142 (assay ID: C_15854163) from Applied Biosystems (Carlsbad, CA) using a Lightcycler 480 Real-Time Polymerase Chain Reaction System (Roche Applied Science, Indianapolis, IN).

**Statistical analysis**

Baseline demographics were summarized using means and SDs, and frequency and percentages as appropriate. It was unclear whether the form of the relationship between allopurinol dose and SU concentration or between allopurinol dose and oxypurinol concentration was linear or log-linear, therefore, we fitted both models to the data for each individual. For ~75% of individuals, the linear association was stronger for both the relationship between allopurinol dose and SU concentration or between allopurinol dose and oxypurinol concentration with median correlation coefficients ρ = 0.70 and 0.66, respectively. For this reason, we calculated the linear slope for both the association between allopurinol dose and SU concentration for each individual as a measure of sensitivity to allopurinol and for the association between allopurinol dose and plasma oxypurinol concentration as a measure of allopurinol metabolism. The univariate associations between factors potentially associated with allopurinol metabolism. The univariate associations between factors potentially associated with allopurinol sensitivity and drug metabolism were tested using Pearson’s correlation coefficients and analysis of variance. The independent associations between factors potentially influencing allopurinol sensitivity and drug metabolism were tested using forward and backward stepwise general linear models. A two-tailed P value < 0.05 was taken to indicate statistical significance.

Receiver operator characteristic (ROC) curves were used to identify oxypurinol concentration predictive of achieving a SU concentration < 6 mg/dL. ROC curves were generated for the group as a whole as well as individual CrCl.
groups (< 30 mL/minute, 30–59 mL/minute, and ≥ 60 mL/minute). Cut points were identified from the ROC curves, which optimized sensitivity and specificity for each CrCL group (Table 3). The sensitivity, specificity, negative predictive value, and positive predictive value for predicting target SU concentration < 6 mg/dL were generated from these cut points.

RESULTS
Baseline characteristics
Of the original 183 participants in the study, 129 (70.5%) had allopurinol dose, SU, and plasma oxypurinol concentrations available at multiple time points over the study period and had genotyping data for ABCG2 rs2231142. Of 129 participants, 113 were men (87.6%), 55 were European (42.6%), 67 were Māori or Pacific Island (51.9%), and 57 were receiving a diuretic at study entry (44.2%). At baseline mean (SD) age was 59.7 years (12.2 years), SU was 7.25 mg/dL (1.46 mg/dL), CrCL was 59.15 mL/minute (27.27 mL/minute), and allopurinol dose was 256 mg/day (102 mg/day). Of the 129 participants, 74 (57.4%) were homozygous for the ABCG2 G (Q, glutamine) allele, 44 (34.1%) had 1 copy of the minor T allele, and 11 (8.5%) were homozygous for the T (K, lysine) allele.

Relationship among allopurinol dose, oxypurinol, and SU concentrations
Using all observations from all individuals, there were a wide range of SU concentrations for each allopurinol dose (Figure 1a). The relationship between sensitivity to allopurinol (change in urate) and allopurinol metabolism (change in oxypurinol) for each 100 mg increase in allopurinol dose was varied between individuals (Figure 1b,c). There was a significant association between sensitivity to allopurinol (change in urate mg/dL) and allopurinol metabolism (change in oxypurinol μmol/L) for each 100 mg increment in allopurinol dose ($r = -0.60; P < 0.001$; Figure 1d).

The sensitivity to allopurinol (change in urate mg/dL) and allopurinol metabolism (change in oxypurinol μmol/L) for each 100 mg increment in allopurinol dose were significantly different across renal function groups with a smaller reduction in urate and smaller increase in oxypurinol in those with better renal function ($P = 0.001; P < 0.001$, respectively, Table 1). However, the association between allopurinol sensitivity (change in urate mg/dL) and allopurinol metabolism (change in oxypurinol μmol/L) did not differ significantly ($y = test comparing slopes P = 0.45$) across the three groups defined by kidney function (Figure 2).

Figure 1 (a) Individual allopurinol doses as associated with a wide range of serum urate concentrations. (b) Variation between individuals in allopurinol sensitivity (change in urate mg/dL) with increasing allopurinol dose. (c) Variation in allopurinol metabolism (change in oxypurinol μmol/L) for each 100 mg increase in allopurinol dose. (d) Relationship between allopurinol metabolism and sensitivity to allopurinol.
Factor associations with allopurinol sensitivity and drug metabolism

Univariate analysis was used to examine a variety of factors potentially associated with allopurinol sensitivity and metabolism, including age, body mass index (BMI), ethnicity, sex, CrCL, baseline urate, and ABCG2 Q141K (Table 2). There was a dose response effect with a significantly larger increase in oxypurinol for each 100 mg increment in allopurinol for those with the ABCG2 GG compared with TT genotype and a corresponding greater reduction in SU concentration for each 100 mg increments in allopurinol dose (Figure 3a, b).

Multivariate models

To determine factors that might be associated with sensitivity to allopurinol, multivariate analysis independent of other measured factors was undertaken. Factors included BMI, age, renal function, baseline urate, sex, ethnicity, and ABCG2 Q141K. BMI (P = 0.023), CrCL (P = 0.037), Q141K (P = 0.019), and baseline urate (P = 0.004) were all associated with allopurinol sensitivity independent of the other factors included in the analysis, such that those with higher BMI, better kidney function, or ABCG2 TT had a smaller reduction in urate for a 100 mg increase in allopurinol dose, whereas those with a higher baseline urate had a larger reduction in urate for a 100 mg allopurinol dose increase (R² = 0.19).

The same four variables were associated with allopurinol metabolism (BMI: P = 0.034; CrCL: P < 0.001; baseline urate: P = 0.014; and ABCG2 TT: P = 0.038; R² = 0.37) independent of the other variables included in the analysis, such

**Table 1 Association between allopurinol sensitivity (change in urate) and allopurinol metabolism (change in oxypurinol) by renal function**

| Renal Function | Allopurinol Sensitivity (change in urate (mg/dL) for each 100 mg increase in allopurinol) | Oxypurinol Metabolism (change in oxypurinol (μmol/L) for each 100 mg increase in allopurinol) |
|----------------|------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| CrCL < 30 mL/minute | -1.34 (0.69)                                                                            | 71.9 (45.7)                                                                               |
| CrCL 30–<60 mL/minute | -1.08 (0.78)                                                                            | 59.9 (38.5)                                                                               |
| CrCL ≥ 60 mL/minute | -0.74 (0.53)                                                                            | 21.9 (23.4)                                                                               |

CrCL, creatinine clearance.

Data presented are mean (SD).

**Figure 2 Relationship between allopurinol metabolism and allopurinol sensitivity by renal function group. CrCL, creatinine clearance.**
that those with better renal function, lower baseline urate, ABCG2 TT genotype, or higher BMI had a smaller increase in oxypurinol for a 100 mg increase in allopurinol dose.

Minimum therapeutic oxypurinol concentration

ROC curves were used to estimate sensitivity, specificity, negative predictive value, and positive predictive value of oxypurinol concentration for predicting target SU concentration < 6 mg/dL for the group as a whole as well as by renal function status (Table 3). The minimum oxypurinol concentration for achieving the urate target was found to be about 104 μmol/L, but predictive accuracy was poor (ROC curve area under the curve (AUC) 0.65). The AUC values of the ROC curves were similar across all 3 CrCL groups, ranging from 0.67–0.73. Those with worse kidney function required higher concentrations of oxypurinol to achieve target urate (Table 3). The optimal minimum concentration for achieving target was strongly associated with CrCL.

DISCUSSION

We have shown that there is wide interindividual variability in response to allopurinol dose escalation with regard to change in SU concentration and change in plasma oxypurinol concentration. Four readily available clinical variables, namely BMI, age, baseline urate, and renal function contribute to the variability of response to allopurinol dose escalation with regard to change in plasma oxypurinol and change in urate concentrations.

We have previously reported that ABCG2 rs2234412 (Q141K) genotype is associated with poor response to allopurinol, defined as SU concentration > 6 mg/dL despite allopurinol > 300 mg/day. The mechanism by which ABCG2 Q141K

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**Table 2 Univariate associations of factors associated with allopurinol sensitivity and allopurinol metabolism**

|                        | Allopurinol sensitivity (change in urate mg/dL for each 100 mg increase in allopurinol) | Allopurinol metabolism (change in oxypurinol μmol/L for each 100 mg increase in allopurinol) |
|------------------------|--------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Sex                    | 2.34 (0.13)                                                                                  | 8.31 (0.005)                                                                                  |
| Ethnicity              | 2.33 (0.08)                                                                                  | 1.63 (0.19)                                                                                  |
| BMI                    | 0.13 (0.14)                                                                                  | −0.10 (0.26)                                                                                  |
| Age                    | −0.25 (0.003)                                                                                 | 0.36 (< 0.001)                                                                               |
| CrCL                   | 0.29 (0.001)                                                                                 | −0.55 (< 0.001)                                                                               |
| Baseline urate         | −0.24 (0.007)                                                                                 | 0.27 (0.002)                                                                                 |
| ABCG2 genotype         | 2.75 (0.07)                                                                                  | 2.89 (0.059)                                                                                 |

BMI, body mass index; CrCL, creatinine clearance.

*aWomen had a greater increase in oxypurinol for each 100 mg increase in allopurinol compared to men (women 69.3 vs. 41.1 μmol/L per 100 mg allopurinol). Data reported as F(df1,df2) or r (p).*

**Table 3 Sensitivity and specificity of minimum oxypurinol concentration to achieve target urate (<6 mg/dL)**

| CrCL                        | ROC AUC (SE) | Oxypurinol minimum concentration (μmol/L) | Sensitivity (%) | Specificity (%) | NPV (%) | PPV (%) |
|-----------------------------|--------------|-------------------------------------------|-----------------|-----------------|---------|---------|
| Entire group (n = 2,219)    | 0.65 (0.01)  | 103.6                                     | 59.5            | 64.2            | 59.3    | 63.4    |
| < 30 mL/minutes (n = 284)   | 0.69 (0.03)  | 181.8                                     | 62.4            | 76.6            | 74.4    | 65.2    |
| 30–< 60 mL/minutes (n = 844) | 0.67 (0.02)  | 172.8                                     | 51.5            | 75.5            | 60.3    | 68.3    |
| ≥ 60 mL/minutes (n = 1,091) | 0.73 (0.02)  | 82.9                                      | 59.5            | 75.7            | 60.7    | 74.8    |

AUC, area under the curve; CrCL, creatinine clearance; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operator curve.
alters allopurinol response remains unclear. ABCG2 has been reported to be an efflux pump for both allopurinol and oxypurinol with the ABCG2 141K variant impairing ABCG2 function resulting in intracellular accumulation of allopurinol and oxypurinol. However, ABCG2 only transports oxypurinol and, thus, dysfunctional variants of ABCG2, such as 141K, would lead to decreased renal excretion of oxypurinol, higher serum oxypurinol levels, and a greater urate-lowering effect. However, we have shown both in cross-sectional data and now in prospective data that the opposite occurs—the 141K allele is associated with a smaller increase in oxypurinol and a smaller urate-lowering effect. This suggests that ABCG2 141K is associated with decreased conversion of allopurinol to oxypurinol or increased renal elimination of oxypurinol, with the latter seeming more likely based on current knowledge of ABCG2 function. These data are consistent with ABCG2 141K being associated with poor response to allopurinol. It is important to note that other genes may be involved in allopurinol response, including GREM2 and GLUT 9, and further research will be required to determine how they might help clinically.

Measurement of plasma oxypurinol concentrations is not currently part of routine clinical care, although measurement may assist in assessing patient adherence with allopurinol therapy. A plasma oxypurinol concentration of 100 µmol/L has been suggested to be the upper end of the therapeutic range, with most people with gout achieving “normal” SU, defined as < 7.6 mg/dL (0.45 mmol/L), at concentrations < 100 µmol/L. More recently, we reported that the majority of people with gout required plasma oxypurinol 100–150 µmol/L to achieve the target SU (< 6 mg/dl; 0.36 mmol/L). Higher oxypurinol concentrations in the more recent studies likely relate to the lower target urate compared with the older studies (6 mg/dl vs. 7.6 mg/dL; and < 0.36 mmol/L vs. < 0.45 mmol/L). Herein, we have shown there is variability in oxypurinol concentration required to achieve target by renal function group with higher concentrations required in those with worse kidney function and that the low sensitivity and specificity of oxypurinol may well preclude its usefulness in therapeutic drug monitoring, other than to assess adherence. However, studies of people commencing allopurinol as well as more complex pharmacokinetic/pharmacodynamic models may be required to determine an accurate therapeutic range. Such models would allow for the impact of covariates on the relationship between oxypurinol and urate lowering, as well as factors such as diuretics and genotype to be explored.

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1. Khanna, D. et al. 2012 American College of Rheumatology Guidelines for the Management of Gout. Part I: systematic nonpharmacologic and pharmacologic therapeutic approaches to hyperuricemia. Arthritis Care Res. 64, 1431–1446 (2012).
2. Richette, P. et al. 2016 updated EULAR evidence-based recommendations for the management of gout. Ann. Rheum. Dis. 75, 29–42 (2017).
3. Stamp, L. et al. Can we predict inadequate response to allopurinol dose escalation? Analysis of a randomised controlled trial. Rheumatology 57, 2183–2189 (2018).
4. Graham, G. et al. Understanding the dose-response relationship of allopurinol: predicting the optimal dosage. Br. J. Clin. Pharmacol. 76, 932–938 (2013).
5. Wright, D. et al. The impact of diuretic use and ABCG2 genotype on the predictive performance of a published allopurinol dosing tool. Br. J. Clin. Pharmacol. 84, 937–943 (2018).
6. Wright, D., Duffull, S., Kerrigan, L., Dabbert, N., Barclay, M. & Stamp, L. Predicting response to allopurinol in patients with gout. Br. J. Clin. Pharmacol. 81, 277–289 (2016).
7. Wallace, M. et al. Association between ABCG2 rs2231142 and poor response to allopurinol: replication and meta-analysis. Rheumatology 57, 656–660 (2018).
8. Peterson, G. et al. Dosage prescribing and plasma oxypurinol levels in patients receiving allopurinol therapy. Eur. J. Clin. Pharmacol. 39, 419–421 (1990).
9. Emerson, B., Gordon, R., Cross, M. & Thomson, D. Plasma oxypurinol concentrations during allopurinol therapy. Br. J. Rheumatol. 26, 445–449 (1987).
10. Stamp, L. et al. Relationship between serum urate and plasma oxypurinol in the management of gout: determination of minimum plasma oxypurinol concentration to achieve a target serum urate level. Clin. Pharmac. Ther. 90, 382–389 (2011).
11. Stamp, L. et al. A randomised controlled trial of the efficacy and safety of allopurinol dose escalation to achieve target serum urate in people with gout. Ann. Rheum. Dis. 76, 1522–1528 (2017).
12. Stamp, L. et al. Allopurinol dose escalation to achieve serum urate below 6 mg/dL: an open label extension study. Ann. Rheum. Dis. 76, 2065–2070 (2017).
13. Stamp, L. et al. Using allopurinol above the dose based on creatinine clearance is effective and safe in chronic gout, including in those with renal impairment. Arthritis Rheum. 63, 412–421 (2011).
14. Wen, C. et al. Genome-wide association study identifies ABCG2 (BCRP) as an allopurinol transporter and a determinant of drug response. Clin. Pharmacol. Ther. 97, 516–525 (2015).
15. Nakamura, M., Fujita, K., Toyoda, Y., Takada, T., Hasegawa, H. & Ichiha, K. Investigation of the transport of xanthine dehydrogenase inhibitors by the urate transporter ABCG2. Drug Metabol. Pharmacokinet. 33, 77–81 (2018).
16. Stamp, L. et al. Genome-Wide Association and Functional Studies Reveal Novel Pharmacological Mechanisms for Allopurinol. Clin Pharmacol Ther. 106, 623–631 (2019).
17. Brackman, D. et al. Genome-Wide Association and Functional Studies Reveal Novel Pharmacological Mechanisms for Allopurinol. Clin Pharmacol Ther. 106, 623–631 (2019).
18. Stamp, L. et al. Plasma oxypurinol as a measure of adherence in clinical trials. Ann Rheum Dis 77, 313–4 (2017).