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GENES AND DISEASE

Genomic sequencing of uric acid metabolizing and clearing genes in relationship to xanthine oxidase inhibitor dose

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Abstract It remains unclear why the dose of xanthine oxidase inhibitors (XOI) allopurinol or febuxostat varies among patients though they reach similar serum uric acid (SUA) goal. We pursued genomic sequencing of XOI metabolism and clearance genes to identify single-nucleotide polymorphisms (SNPs) relate to differences in XOI dose. Subjects with a diagnosis of Gout based on the 1977 American College of Rheumatology Classification Criteria for the disorder, who were on stable doses of a XOI, and who were at their goal SUA level, were enrolled. The primary outcome was relationship between SNPs in any of these genes to XOI dose. The secondary outcome was relationship between SNPs and change in pre- and post-treatment SUA. We enrolled 100 subjects. The average patient age was 68.6 ± 10.6 years old. Over 80% were men and 77% were Caucasian. One SNP was associated with a higher XOI dose: rs75995567 ($p = 0.031$). Two SNPs were associated with 300 mg daily of allopurinol: rs11678615 ($p = 0.022$) and rs3731722 on *Aldehyde Oxidase* (*AO*) (His1297Arg) ($p = 0.001$). Two SNPs were associated with a lower dose of allopurinol: rs1884725 ($p = 0.033$) and rs34650714 ($p = 0.006$). For the secondary outcome, rs13415401 was the only SNP related to a smaller mean SUA change. Ten SNPs were identified with a larger change in SUA. Though multiple SNPs were identified in the primary and secondary outcomes of this study, rs3731722 is known to alter catalytic function for some aldehyde oxidase substrates.

Keywords Gout · Pharmacogenomics · Xanthine oxidase inhibitor

Introduction

Gout is a type of inflammatory arthritis resulting from the deposition of monosodium urate (MSU) crystals in joints and in other periarticular sites. A persistently elevated serum uric acid (SUA) level is essential for the formation of MSU crystals [1]. Typical clinical features of gout are acute attacks of synovitis most frequently involving the first metatarsophalangeal joint; however, attacks in other peripheral joints and in the bursa are also common. Oligoarticular and polyarticular forms of the disease have also been recognized. If untreated or poorly controlled, gout can become disabling [1]. In the 1960s, the first xanthine oxidase inhibitor (XOI), allopurinol, was approved for use by the United States Food and Drug Adminstration (FDA). It is a purine analogue which interrupts uric acid synthesis by impairing the conversion of hypoxanthine to xanthine and then ultimately to uric acid [2]. Over the last few decades, allopurinol has demonstrated reliable long-term reduction in SUA levels and quite effectively controls manifestations of gout. In 2009, the FDA approved febuxostat, the second XOI available to control the manifestations of gout [2]. While its use tends to be restricted more to patients with chronic kidney disease or those intolerant of allopurinol, it

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Table 1 Classification criteria for acute gout [13]

Monosodium urate monohydrate microcrystals in joint fluid during an attack
Tophus (proven or suspected)
6 or more of the 11 criteria listed below:
More than 1 attack of acute arthritis
Monoarthritis attack
Maximum inflammation developing within 1 day
Redness observed over joints
1st metatarsophalangeal (MTP) joint pain or swelling
Unilateral 1st MTP joint attack
Unilateral tarsal joint attack
Hyperuricemia
Asymptomatic swelling within a joint on X-ray
Subcortical cysts without erosions on X-ray
Joint fluid culture negative for organisms during attack

is a potent nonpurine analogue XOI [2]. It is also an effective medication in providing long-term control of the manifestations of gout [3].

Despite the availability of effective XOI for the management of gout, the majority of patients on one of the two XOI do not reach a goal SUA [4]. While some of this failure to reach a goal SUA is based on the previously suggested relationship between doses of allopurinol over 300 mg a day in patients with renal impairment and the development of the allopurinol hypersensitivity syndrome (AHS) [5], there is no clear evidence to support using reduced doses of allopurinol to reduce the risk of AHS [6, 7]. Also, while allopurinol has been approved by the FDA for a dose up to 800 mg a day, the vast majority of prescriptions are written for doses $\leq$300 mg a day [7]. Variability in allopurinol dose to achieve a goal SUA remains somewhat unexplained, but as in the case of the AHS, reactions are not always dose dependent and do not always correlate with serum oxypurinol levels [7]. For febuxostat, the pharmacokinetics are linear for doses between 10 mg and 120 mg daily. Across this range, febuxostat sustainably reduces SUA by 25–70% regardless of whether uric acid is underexcreted or overproduced [8].

Single-nucleotide polymorphisms (SNPs) in the xanthine oxidase gene (*XO*) appear to relate to medical conditions such as hypertension, atherosclerosis, and chronic kidney disease [9]. In 2008 Kudo et al. [10] studied 96 Japanese adults describing various *XO* SNPs along with the enzyme kinetics of these polymorphisms in vitro. From this study, two *XO* SNPs associated with a higher functioning enzyme were identified: 2107A > G (rs17011368) on Exon 20 and a novel polymorphism 3662A > G on Exon 34. These SNPs are associated with approximately a two-fold higher activity than wild-type *XO* [10]. While our pilot study identified a dose relationship between the presence of the *XO* 2107A > G SNP [11] and allopurinol dose, this relationship was not confirmed when tested again with a second, larger cohort of gout patients[Carroll MB, data not published]. Another study investigated whether or not the aldehyde oxidase (*AO*) SNP 3404A > G (rs55754655) and molybdenum cofactor sulfurase (*MOCOS*) SNP 2107C > A (rs594445) were related to those who were "responders" (reached a goal SUA) with allopurinol as compared to "non-responders." [12] While no relationship between either of these two SNPs and "responders" versus "non-responders" was identified, the sample size tested was small and thus a false negative test result was possible [12].

It remains a clinically relevant issue to understand why different patients may require varying doses of XOI but all will reach a similar goal SUA. With effective and safe long-term therapies for gout available, all patients should be safely titrated to a dose which will reliably achieve an appropriate goal SUA. It is reasonable to entertain that some of the variation in XOI dose relates to genetic differences among different subjects. Considering this, we hypothesized that SNPs in genes related to XOI metabolism and clearance were potentially responsible for the variation in XOI dose needed to achieve a goal SUA.

Methods

Study recruitment

We enrolled subjects who were 18 years of age or older who had an established diagnosis of gout, who were on stable dose of their XOI, and who had at least one post-treatment SUA at goal. Subjects were recruited from the Internal Medicine (IM) or affiliated IM Specialties Clinics, the latter in which the Rheumatology Clinic belongs. All patients were outpatients at the time of enrollment and in stable health with no acute medical conditions. Gout was defined according to the 1977 American College of Rheumatology (ACR) classification criteria for the disorder, with a diagnosis being made if they had monosodium urate crystals in synovial fluid aspirated from an affected joint, the presence of a tophus, or satisfied at least 6 of the 11 remaining clinical criteria, of which one was hyperuricemia, as shown in Table 1 [13]. The updated 2015 Classification Criteria for Gout were not used as all subjects were enrolled and data analysis was performed prior to the release of this update. Information about the subject's gout and their XOI use and dose was obtained through direct interview and review of our hospital's electronic medical records. Our electronic medical record provided additional demographic information as well as clinical notes, laboratory studies, and radiographic studies.

Subjects were excluded if they had medical conditions which predisposed them to overproduction of uric acid, specifically if they had a myeloproliferative disorder (treated or untreated) or were actively receiving treatment for neoplasia, more than 5% body surface area affected by psoriasis, or if they consumed more than 14 alcoholic beverages a week. Subjects who were pregnant or nursing were excluded as well. Finally, subjects who were on a XOI but did not have stable SUA values or were not at a goal SUA despite taking their XOI were excluded.

To obtain as accurate a serologic "picture" as possible of the patient's pre-XOI treatment hyperuricemia and their post-XOI treatment achieving a goal SUA, we recorded up to 3 pre- and 3 post-SUA results when available. We queried our electronic medical records for over 15 years of SUA results and XOI prescriptions filled as well as 8 years of clinical encounters. The latter helped screen for acute flares of gout that could have impacted the SUA result recorded, and these values were excluded as they did not represent an equilibrium state. A similar approach was used for calculating the average post-treatment SUA once on XOI. Treatment goals for subjects enrolled in this study were in accord with those delineated by the 2012 ACR Guidelines for the Management of Gout [3]. If no tophi were noted on examination or interview, the goal SUA was $\leq$6 mg/dL; however, if tophi were present, the goal SUA was pushed lower to $\leq$5 mg/dL [3].

Gene selection

Genomic sequencing refers to non-Sanger-based high-throughput DNA sequencing technologies which enable millions of DNA strands to be sequenced in parallel, generating significantly more throughput. We utilized this DNA sequencing technology to screen for single-nucleotide substitutions, insertions, or deletions smaller than 8–10 nucleotides. We studied 6 genes that we thought were most likely to be involved in XOI metabolism or clearance. The first gene was *XO* as the protein xanthine oxidase was the therapeutic target of both XOI. The second gene was *AO* as aldehyde oxidase accounts for a significant amount of the conversion of allopurinol to oxypurinol [14]. The third gene was *MOCOS* as molybdenum cofactor sulfurase is responsible for the incorporation of a molybdenum cofactor in purine oxidases such as aldehyde oxidase and xanthine oxidase. Defective purine oxidases due to SNPs in *MOCOS* would plausible alter allopurinol metabolism, similar to Type II xanthuria. The fourth gene was *SLC22A12* as the urate transport 1 (URAT1) protein which it encodes is responsible for the renal reabsorption of oxypurinol [15]. The last two genes were uridine disphosphate (UDP) glucuronosyltransferase (UGT) 1 family, polypeptides A1 and A4. Both *UGT1A1* and *UGT1A4* encode proteins which catalyze the addition of glucuronic acid to febuxostat and thus participate in the metabolism of this XOI.

Sequence analysis

Prior to alignment, standard FASTQs created for each specimen (following demultiplexing using fastq-multx1) were processed using fastq-mcf1 [ver. EA-Utils 1.04.773: -p 5 -q 7 custom-adapter-file]. The clipped fastqs were then aligned to hg19 using BWA2-MEM [ver. 0.7.3: –l 12] in paired-end fashion to produce binary alignment map (BAM) files for each specimen. The resulting BAMs were used to create pileup files, using samtools3 mpileup [ver 0.1.19: -d 999999 -B -Q 0]. These pileup files were used to call variants as well as calculate depth of coverage statistics. A custom script, parseGenomePileup (v12) was used to provide estimates of coverage, depths, uniformity, and percent bases on target. Variant calling for both single-nucleotide variants (SNVs) and indels was performed with VarPROWL4 (r20278), which incorporates estimates of the per-base sequencing error rate, and the uncertainty around this estimate, along with a list of context-specific sequence motifs known to cause sequencing errors. For every position, VarPROWL estimates the likelihood that the position contains only the reference alleles within the target as a null hypothesis. This test yields a conservative (overestimated) p value, which corresponds to the likelihood that the position is only reference alleles, and a bidirectionality ratio, which corresponds to the symmetry of allele detection on both DNA strands. The bidirectionality ratio was calculated using the absolute value of the log (base 10) transformed ratio of the variant frequencies of both the forward and reverse strands. For a position to be called a variant, the variant frequency of the forward strand must be less than twice the frequency of the reverse strand (and vice versa), which is equivalent to having the bidirectionality ratio be less than 0.3. Positions with low p values ($p < 0.001$) and low bidirectionality ratios (bi-dir < 0.3) were considered to have a variant. Copies of parseGenomePileup and VarPROWL can be provided by Expression Analysis (EA). Annotations of these variants were accomplished with a custom annotation program, chrdex_annotator, which can use standard flat file annotation (such as what is available from UCSC, 1000 Genomes, dbSNP) by creating indexes using the Tidx module in perl [Text::Tidx ver 0.92] to match genomic positions of variants with those with each flat file.

Statistical analysis

The primary study outcome was to identify any relationship between variations in the genetic code related to the metabolism and clearance of XOI, specifically focusing on

single-nucleotide polymorphisms (SNPs), to the XOI dose that our subjects were on. Subjects prescribed allopurinol were divided into three groups: a low-dose group (less than 300 mg daily), a standard dose group (300 mg daily), and a high-dose group (greater than 300 mg daily). To enhance the robustness of the relationship between XOI dose and SNPs, we considered subjects on febuxostat 40 mg daily to be equivalent to allopurinol 300 mg daily (standard dose group), and those subjects prescribed febuxostat 80 mg daily to be equivalent to allopurinol greater than 300 mg daily (high-dose group) [16]. The secondary study outcome was to identify SNPs in the genetic code related to XOI metabolism and clearance to the magnitude of change in SUA. The magnitude of change for those subjects with the SNP was relative to the magnitude of change observed in the wild-type group.

With the outcome of this study to estimate the percentage of patients who would have a specific SNP related to XOI dose, and since there was no available information regarding specific SNPs for our target population, we assumed the chance of a particular SNP being related to a particular XOI dose would be 50%. Our sample size was estimated using the method by Lwanga and Lemeshow [17]. A sample size of 97 patients was calculated. We then enrolled 100 patients at large in our health care system who satisfied the inclusion criteria and had none of the exclusion criteria. Continuous demographic variables were descriptively shown as mean ± standard deviation. Frequency distributions of nominal demographic variables were calculated and graphed. Chi-square testing for 2 × 3 contingency table with exact test of Monte Carlo approach was used. The 95% confidence interval of mean difference and power were analyzed. All the data were analyzed with IBM SPSS Statistics 22 and IBM SPSS SamplePower 3. We chose a level of significant difference of 0.05.

The study was approved by our institutional review board at our medical facility, and written informed consent was obtained from all the study participants prior to enrollment.

Results

Demographic and comorbidity data of our cohort are summarized in Table 2. The average age of the cohort was 68.6 ± 10.6 years old, with over 80% men. Over 75% were Caucasian, and the vast majority had hypertension and hyperlipidemia. Forty-six percent of the cohort had diabetes and 39% had chronic kidney disease with a mean modification of diet in renal disease (MDRD) estimated Glomerular filtration rate (GFR) of 62.7 ± 23.1 cc/min. The average body mass index (BMI) was 31.2 ± 6.2 kg/m^2.

Table 2 Demographic and comorbidity information

	Cohort ($n = 100$)
Age—years old	68.6 ± 10.6
Male gender—no.	81
Ethnicity—no.	
African American	20
Asian	2
Caucasian	77
Pacific Islander	1
Comorbidities—no.	
Hypertension	92
Hyperlipidemia	81
Diabetes mellitus	46
Impaired fasting glucose	7
Atherosclerotic coronary artery disease	26
Chronic kidney disease	39
MDRD calculated GFR (cc/min)	62.7 ± 23.1
Average serum creatinine (mg/dL)	1.2 ± 0.7
History of Cancer[a]	14
BMI (kg/m^2)	31.2 ± 6.2
Medication use—no.	
Diuretics[b]	46
Aspirin ≤325 mg daily	61
Amlodipine	29
Losartan	14
Fenofibrate	3
Aspirin > 325 mg daily	1

BMI body mass index, *MDRD* modification of diet in renal disease, *GFR* glomerular filtration rate

[a] A history of cancer did not include a history of treated or untreated myeloproliferative disorders, leukemia, or lymphoma. Patients with a history of cancer were also not actively receiving treatment at the time of enrollment

[b] Included active use of loop and/or thiazide diuretics at the time of enrollment. Patients receiving Hyzaar (losartan-hydrochlorothiazide combination) were documented as taking both losartan and a diuretic

Nearly half of the cohort was using a diuretic and over 60% were taking aspirin less than or equal to 325 mg daily.

Table 3 provides a summary of the clinical and serologic findings that satisfied the 1977 ACR Classification Criteria for gout. All subjects in this cohort had hyperuricemia, with 98 satisfying the 1977 ACR Classification Criteria for gout. Twelve had tophi noted on exam, and 12 had monosodium urate crystals noted on light microscopy during an acute attack. Over 90% satisfied 6 of the 11 other clinical and serologic criteria of the 1977 ACR Classification Criteria. All patients were actively receiving therapy with a XOI at the time of enrollment—79 prescribed allopurinol and 21 prescribed febuxostat. The distribution of allopurinol dose is noted in the Fig. 1. Subjects in this

Table 3 Clinical characteristics of gout ($n = 100$)

Hyperuricemia—no.	100
Gout—no.	98
Monosodium urate crystals in joint fluid during attack	12
Proven tophus	12
Satisfied at least 6 of the 11 American College of Rheumatology Classification Criteria for gout	93
Patients on XOI—no.	100
On allopurinol	79
On febuxostat	21
Mean pre-treatment SUA (± SD) (mg/dL)*	9.1 ± 1.1
Mean post-treatment SUA (± SD) (mg/dL)	5.0 ± 1.0
Mean change in SUA (± SD) (mg/dL)	4.2 ± 1.4

XOI xanthine oxidase inhibitor (either allopurinol or febuxostat), *SUA* serum uric acid, *SD* standard deviation

* Difference between mean pre- and post-treatment SUA was statistically significant ($p < 0.001$)

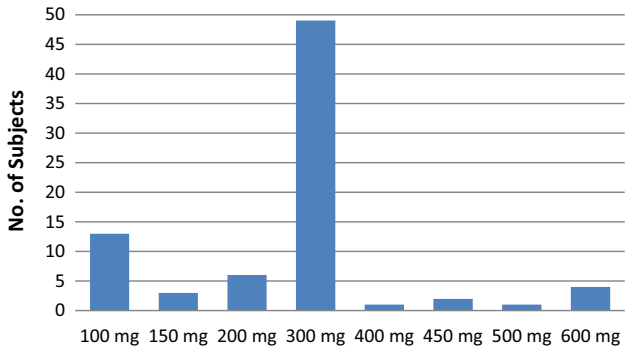

Fig. 1 Allopurinol dose distribution (with Allopurinol dose on 'x' axis)

cohort received between 100 mg and 600 mg of allopurinol daily or if they were treated with febuxostat either 40 mg or 80 mg daily. Almost 50% of the cohort was prescribed allopurinol 300 mg daily at the time of enrollment. Average (±standard deviation) pre-treatment SUA for the cohort was 9.1 ± 1.1 mg/dL, and average post-treatment USA was 5.0 ± 1.0, with an average change in uric acid of 4.2 ± 1.4 mg/dL. The difference between the average pre- and post-treatment SUA was statistically significant ($p < 0.001$).

Regarding the primary outcome of presence of SNP as related to XOI dose, Table 4 summarizes our findings. We identified 5 SNPs that had a relationship to XOI dose. Two SNPs, rs11678615 and rs3731722, were associated with a XOI treatment dose equivalent to allopurinol 300 mg daily. One SNP, rs75995567, was associated with a XOI treatment dose equivalent to allopurinol greater than 300 mg daily. Another SNP, rs1884725, was associated with a XOI treatment dose equivalent to allopurinol 300 mg daily or less. The last SNP, rs34650714, was related to a XOI treatment dose equivalent to allopurinol less than 300 mg daily. The association of all SNPs in regard to their respective XOI dose was adequately powered (power for each >0.98). Of the 5 SNPs, only rs3731722 was a nonsynonymous codon, leading to a change from histidine to arginine at amino acid position 1297 in aldehyde oxidase. This

Table 4 SNPs in relationship to XOI dose (primary outcome)

Reference SNP	Gene	Intron or exon	Nucleotide change	Amino acid change	Cohort MAF (± 95% CI)	dbSNP MAF*	Allopurinol dose[a]	*p*
rs11678615	*AO*	Exon	3987C > T	Val1329=	0.12 ± 0.05	0.1162	300 mg/d	0.025
rs3731722	*AO*	Exon	3890A > G	His1297Arg	0.14 ± 0.06	0.0843	300 mg/d	0.001
rs75995567	*AO*	Intron	T > C	–	0.02 ± 0.02	0.014	>300 mg/d	0.031
rs1884725	*XO*	Exon	3030A > G	Phe1010=	0.88 ± 0.06	0.2001	300 mg/d or less	0.035
rs34650714	*UGT*	Intron	C > T	–	0.07 ± 0.03	0.0425	<300 mg/d	0.007

All associations were adequately powered (power >0.98). No homozygous variants were observed

AO aldehyde oxidase, *XO* xanthine oxidase, *UGT* uridine disphosphate (UDP) glucuronosyltransferase (UGT) 1, *MAF* minor allele frequency, *95 % CI* 95% confidence interval, '='—synonymous codon (no amino acid change)

* dbSNP MAF accessed using website http://www.ncbi.nlm.nih.gov/snp/with data from 1000 Genomes

[a] "Allopurinol dose" refers to the mean allopurinol dose to which the variant gene corresponded

Table 5 Reference SNPs in relationship to SUA change

Reference SNP	Gene	Intron or exon	Nucleotide change	Amino acid change	Cohort MAF	dbSNP MAF	Change in SUA[a] (95% CI) (mg/dL)	*p*
rs28898617	*UGT*	Intron	A > G	Gln6Arg	0.01	0.0098	5.1 (2.2–8.0)	0.001
rs36208390	*XO*	UTR 5′	G > C	–	0.02	0.0138	4.1 (2.0–6.2)	<0.001
rs4148327	*UGT*	Intron	T > C	–	0.01	0.0062	5.1 (2.2–8.0)	0.001
rs3825017	*SLC22A12*	UTR 5′	C > T	Asn82=	0.03	0.0707	2.2 (0.4–4.0)	0.018
rs45511600	*XO*	Intron	G > A	–	0.03	0.0210	2.6 (0.8–4.4)	0.005
rs45604135	*XO*	Exon	G > A	Gly1028=	0.03	0.0196	2.6 (0.8–4.4)	0.005
rs45612738	*XO*	Exon	C > T	Gly378=	0.03	0.0210	2.6 (0.8–4.4)	0.005
rs45612839	*XO*	Exon	C > G	Gly503=	0.09	0.0627	1.3 (0.2–2.4)	0.018
rs59942447	*MOCOS*	Intron	G > A	–	0.16	0.1288	0.9 (0–1.8)	0.044
rs75995567	*AOX1*	Intron	T > C	–	0.02	0.0140	2.4 (0.2–4.6)	0.037

The association of larger SUA change with rs36208390, rs45511600, rs45604135, and rs45612738 was adequately powered (power >0.8). No homozygous variants were observed

SUA serum uric acid, *AO* aldehyde oxidase, *XO* xanthine oxidase, *MOCOS* molybdenum cofactor sulfurase, *UGT* uridine disphosphate (UDP) glucuronosyltransferase (UGT) 1, *SLC22A12* solute carrier family 22 (organic anion/urate transporter), Member 12, *UTR 5′* untranslated region 5′, *MAF* minor allele frequency, *95 % CI* 95% confidence interval

* dbSNP MAF accessed using website http://www.ncbi.nlm.nih.gov/snp/with data from 1000 Genomes

[a] The value listed in "Change in SUA" is the difference between the mean SUA for those with the variant and the mean SUA for those with the wild type

variant (rs3731722) was in Hardy–Weinberg equilibrium ($p = 0.4516$) with 86 wild types and 14 heterozygotes (no homozygotes identified). The other SNPs identified in this study were either synonymous codon changes (rs11678615 and rs1884725) or intronic.

For the secondary outcome, exploring relationships of SNPs to change in SUA, rs13415401, was the only SNP that we found which was related to a smaller change in SUA as compared to the wild type. The mean change (±95% CI) in SUA with this SNP was 1.5 ± 1.0 mg/dL. The relationship was adequately powered (power > 0.8). Ten SNPs were identified as leading to a larger change in SUA as compared to the wild type. These SNPs are summarized in Table 5. Most of the SNPs identified in the secondary outcome analysis were either intronic or synonymous codon changes in exon regions of *XO*. One only SNP, rs28898617, was related to a nonsynonymous codon change in an intron region. The relationships between the SNPs rs36208390, rs45511600, rs45604135, and rs45612738 with a larger mean change in SUA were adequately powered (power >0.8).

Discussion

Gout is a common medical condition which can be challenging for both patients and their providers to adequately control, even in an era where effective long-term therapy (XOI) exists [18]. With the prevalence of gout increasing throughout the developed world [18, 19] and with less than half of patients on XOI therapy achieving treatment goals, the issue of treatment failure in gout will continue to be a significant medical issue [19]. When compliant with XOI therapy, we have noted in our practice that XOI therapy is not "one size fits all," as patients reach their goal SUA on a variety of doses. While this variability could be due to factors such as decreased conversion of allopurinol to oxypurinol, increased renal excretion of oxypurinol, abnormality in XO structure and/or function rendering oxypurinol less effective, and/or drug interactions, we hypothesized that genetic variability in the metabolism and clearance of XOI might explain variations in XOI dosing [18]. While the results of our primary outcome identified 5 SNPs related to XOI dose, based on our current understanding of the significance of SNPs on protein structure and function, rs3731722, which leads to a nonsynonymous codon change in aldehyde oxidase, is the most intriguing. Aldehyde oxidase is a molybdoflavoprotein that is homologous with xanthine oxidase, and given the similarity in structure, the properties of the 2 enzymes are very similar [20, 21]. Aldehyde oxidase metabolizes allopurinol like xanthine oxidase does [14]. The SNP rs3731722 is near amino acid 1270, the site of a proton acceptor related to aldehyde oxidase enzymatic activity [22]. Histidine is a medium-sized, polar amino acid and arginine is a large size, polar amino acid [23]. While it has been reported that histidine is often found in protein-active sites and does not substitute well [23], the web-based amino acid substitution prediction tools PolyPhen and SNPs3D both suggest the nonsynonymous codon of rs3731722 is benign/harmless [24, 25].

Studies have, however, identified enhanced conversion of some substrates by aldehyde oxidase with this SNP, so the relationship that we identified between this SNP and the dose of XOI could have biologic plausibility [21, 26, 27]. What remains unclear is how the presence of rs3731722 would decrease the variation in XOI dose as those with the SNP reached their goal with a standard dose of allopurinol. Regarding the secondary outcome, while multiple SNPs were identified as having a relationship to magnitude of change in SUA, current limitations in our knowledge of SNPs in introns or untranslated regions prevent a meaningful supposition of how the SNP leads to a clinically meaningful change.

The data that we present in this study should be viewed in the context of several limitations of this study. First, the study was genomic sequencing study and not a genome wide association study (GWAS), so while the SNPs that we identified are of interest, we did not have the larger sample size or statistical power than make the results of GWAS more authoritative. For example, a recent article identifying a relationship between rs2231142 and a reduced response to allopurinol was the first GWAS to assess allopurinol response [28]. The SNP rs2231142 is a nonsynonymous codon in the *ABCG2* gene which encodes the transporter Breast Cancer Resistance Protein which therefore would not have initially been hypothesized revealing the advantage of the GWAS [28]. No SNPs were identified in this study in the genes responsible for metabolism or clearance of XOI [28]. The GWAS by Wen et al. should be considered more informative about future research directions in gout genetics and poor response to XOI. Second, this study was not designed to test molecular effects should nonsynonymous codons be identified. This again is a strength of the Wen et al. [28] study which conducted cellular isotopic uptake studies and demonstrated that genetic variants such as rs2231142 affected allopurinol transport. Third, most of the SNPs in this study were either synonymous codons or introns. While synonymous codons would not lead to a structural or functional change in the protein, it is unclear what change the SNPs in introns would have. Fourth, the minor allele frequencies identified in our study varied with those provided in the dbSNP 1000 Genomes database [29]. This would be expected given the modest size and relative heterogeneity of this study; however, the minor allele frequency provided by the dbSNP 1000 Genomes database was within the 95% confidence interval for 4 of the 5 alleles identified in this cohort. Only one SNP, rs1884725, was present at a frequency of 0.88 in this cohort, whereas in the dsSNP 1000 Genomes the frequency was significantly lower and statistically different from the frequency of 0.2001. Fifth, our study had a handful of subjects prescribed more than 300 mg a day of allopurinol. Clinicians are typically comfortable prescribing allopurinol up to 300 mg a day, but doses beyond this make cause consternation given the persistence of the now refuted concern that doses over 300 mg a day increase the risk for the allopurinol hypersensitivity syndrome [5, 30]. We had intended to recruit a more robust subgroup in our cohort who were routinely on more than 300 mg a day of allopurinol to see if a particular SNP was related to increased metabolism or clearance, but few patients were noted to be on these doses. Sixth, our study design and the selection of the patients likely introduce some degree of bias and thus the results may not be as generalizable as other studies, again such as a GWAS study. Finally, as demonstrated by Wen et al. [28], it is possible that XOI metabolism and clearance genes factor little (if at all) into dose of XOI needed to reach a goal SUA whereas non-XOI metabolism or clearance genes are actually more appropriate targets for further study.

Despite our study limitations, our study displays several strengths. First, we were fortunate to have an electronic medical record that provided SUA data for most subjects going back more than 15 years. The ability to screen many SUA values helped provide a reasonable average of pre- and post-treatment SUA and thus delta SUA for our secondary outcome. Second, our subjects were uniformly treated to national/international goals and thus provide a picture of what the genetic background might be in a group compliant with XOI therapy and at a recognized goal. Third, our study continues to add to our understanding of the genetics responsible for the metabolism and clearance of XOI. While rs3731722 is intriguing in regard to the possible change in protein structure/function that it imparts to XOI dose, all of the SNPs in this study suggest that at this time genetics related to XOI metabolism and clearance may not be as important as non-XOI metabolism or clearance SNPs [28]. Finally, our study continues to enhance our knowledge regarding a significant clinical issue that still requires clarification, specifically why different patients need different doses of XOI to reach their goal.

In conclusion, we designed this study to investigate whether or not genetic variants, specifically SNPs, affecting proteins that metabolize or clear XOI were related to the dose needed to achieve a goal SUA. While our cohort was heterogeneous and modest in size, we did identify multiple introns and synonymous codons which were related to dose of XOI needed and magnitude of change in SUA. Given the limitations in our current understanding of intron and untranslated region SNPs, the SNP rs3731722 in *AO* might be clinically significant given the histidine to arginine conversion that occurs as a consequence. Future cellular or molecular studies might be helpful in determining if this protein change is truly clinically significant in the management of gout. As elegantly summarized by Stamp et al. [18], a clinically based, multi-variable model which also incorporates “genes that influence serum urate and

allopurinol metabolism, excretion or mechanism of action may guide the clinician in choosing the most appropriate first-line urate-lowering therapy." We strongly concur with her future vision of how clinicians will hopefully one day choose and dose XOI, but would suggest that future genetic work focus on genes beyond those traditionally associated with purine metabolism and clearance.

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Compliance with ethical standards

Conflict of interest Author Carroll, MB declares that he has no conflict of interest. Author Smith, DM declares that he has no conflict of interest. Author Shaak, T declares that he has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent The voluntary, fully informed consent of the subjects used in this research was obtained as required by 32 CFR 219 and AFI 40-402, Protection of Human Subjects in Biomedical and Behavioral Research. Informed consent was obtained from all individual participants included in the study.

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