The Effects of BCRP Q141K on Allopurinol Pharmacokinetics and Dynamics

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PURPOSE: To investigate the potential association of polymorphic genetic variants ABCG2 with altered response to allopurinol, we will employ a genotype to phenotype strategy. Specifically, we will evaluate this hypothesis in Asians and Europeans, populations with rapidly growing incidence of hyperuricemia and gout that carry these genetic variants. To assess the effects of these variants on allopurinol response, we will measure serum uric acid and renal uric acid clearance as well as serum and urinary concentrations of allopurinol and its active metabolite, oxypurinol, to determine pharmacokinetic parameters. Other analyses may be done in the calculation of these parameters, such as serum creatinine and creatinine clearance.

BACKGROUND:

1. Gout is a major medical condition with a high prevalence in the U.S. and an increasing prevalence in global populations. Gout is an inflammatory arthritic disease caused by elevated serum uric acid (UA) levels. In humans, UA is the end-product of purine metabolism, and high UA levels are associated with gout and increased risk for cardiovascular events including stroke and myocardial infarction. Historically, because of protein-rich diets, gout was termed a "rich man's disease" and confined to Western populations. However, with the global epidemic of metabolic syndrome, the prevalence of gout in other populations has increased dramatically[1,2], e.g., certain populations in Asia, Polynesia and Africa have prevalence rates exceeding 10%[3,4].

2. Allopurinol (ALLO) is first line therapy for gout prevention with emerging uses in other diseases associated with metabolic syndrome. Discovered in the 1940s by researchers at Burroughs Wellcome ,including Nobel laureates Elion and Hitchings, ALLO has been first-line therapy for the treatment of hyperuricemia and gout for many years. ALLO is considered a pro-drug with weak pharmacologic effects; it is converted to an active metabolite, oxypurinol (OXY) via aldehyde oxidase, AOX1. OXY is a potent inhibitor of xanthine oxidase, the primary enzyme involved in the synthesis of UA. Though its major use is in the treatment of gout, ALLO is used for other diseases such as tumor lysis syndrome and kidney stones[5-7], and is being repurposed for the treatment of diseases associated with metabolic syndrome, e.g., heart failure, stroke and cardiac shock[8-11]. Other xanthine oxidase inhibitors are also on the market or in clinical drug development.

3. Recent data from our laboratory challenge the widely accepted mechanism of action of ALLO and OXY as its sole mechanism and suggest a major role for BCRP. In the first GWAS of ALLO response, we observed a single locus, in ABCG2, strongly associated with response to ALLO in 1300 patients of European ancestry. Previous genomewide association studies have shown ABCG2 and other UA transporters (e.g. GLUT9, URAT1) to be associated with high UA levels[12-15]. However, in our population of gout patients on ALLO, only ABCG2 (and not the other UA transporters) was significantly associated with drug response, i.e., ALLO-induced changes in UA levels. These results suggest that the gene may act on OXY directly. In fact, in exciting follow-up studies in our laboratory, we found that both ALLO and OXY are excellent substrates of ABCG2 in HEK293 cells over-expressing the transporter suggesting that ABCG2 may

function directly in the pharmacokinetics of ALLO/OXY. These results are consistent with other pharmacogenomic studies that show that genetic variants in membrane transporters that affect pharmacokinetics are associated with altered drug response (e.g., SLCO1B1 and methotrexate and statin toxicities, and ABCG2 and rosuvastatin response)[16-19]. Because of its location on the apical membranes of intestinal and renal epithelia, the data suggest that ABCG2 functions in reducing the intestinal absorption and enhancing the renal secretion of the drugs as it does for several other drugs[20-22].

4. A common reduced function variant of ABCG2, Q141K, paradoxically leads to reduced response to ALLO. The ABCG2 locus identified in our GWAS included the nonsynonymous variant, Q141K (rs2231142), which is known to have reduced transport function due to reduced protein expression[23,24]. This variant had the lowest p-value of all ABCG2 nonsynonymous variants that were either genotyped or imputed. Interestingly, Q141K has also been associated, at genomewide level significance, with response to rosuvastatin[19]. Because our preliminary studies indicate that ABCG2 is an efflux transporter for OXY and ALLO, we expected that the variant allele would be associated with higher drug levels through increased absorption and reduced renal secretion mechanisms resulting in higher drug levels and improved response. The fact that we found that the variant was associated with a worse response suggests another mechanism. We propose that Q141K results in lower drug levels in the renal tubule, consistent with reduced renal secretion of the drugs. The lower levels of OXY in the renal tubule result in reduced inhibition of UA reabsorptive transporters through competitive inhibition mechanisms. In support of our hypothesis, we recently found that OXY and ALLO inhibit UA uptake via URAT1. Our data are consistent with previous studies showing that OXY is a substrate of URAT1[25]. Also, reviewing data in the older literature we noted that ALLO treatment was associated with an increased renal clearance of UA in many patients after initiation of ALLO treatment[26], supporting our hypothesis that ALLO and/or OXY inhibit UA reabsorptive transporters. Therefore we propose that ABCG2-mediated secretion of OXY results in sufficiently high levels of the drug in the renal tubule to competitively inhibit uric acid reabsorptive transporters, e.g., URAT1. Competitive inhibition of uric acid reabsorption leads to increased UA excretion and therefore reduced serum levels of UA. Notably, ABCG2 can concentrate its substrates >20-fold[20,27] and maximum plasma unbound concentrations of OXY and ALLO are as high as 100 μM. Therefore, estimated proximal tubule concentrations of OXY and ALLO could be in the mM range[28]. In contrast, lower tubule levels of OXY, which are achieved in individuals with the Q141K variant, result in less inhibition of UA reabsorption and therefore, poorer response. If our hypotheses are proven, our studies will lead to a new understanding of the mechanisms of action of ALLO and its active metabolite OXY.

5. There is a major gap in our understanding of the genetic factors that determine response to OXY in all ethnic groups including under-represented populations. In contrast to the multiple GWAS studies identifying genetic risk factors for hyperuricemia and gout, there has been no published GWAS related to ALLO response. In preliminary studies using samples from individuals of European ancestry, we conducted the first GWAS of response to ALLO. We attempt to fill this gap by evaluating the effects of ABCG2 gene variants on the pharmacodynamics and pharmacokinetics of ALLO. This effort is a mechanistic genotype to phenotype pharmacogenetic study designed to supplement ongoing investigations of the contribution of genetic variability in drug

transporters to inter-individual variation in drug response. Comparing the variability in uric acid levels and renal clearance of ALLO in individuals with different ABCG2 genotypes will provide information about the extent to which genetic factors contribute to inter-individual differences in the renal elimination and response to ALLO. Investigating these polymorphic variants, which occur at relatively high frequencies within Asians and Europeans, will provide vital information for the purpose of guiding antihyperuricemic drug regimens in these populations.

NUMBER OF SUBJECTS: 15 subjects will participate in the study. 5 from each genotype: homozygous recessive, homozygous dominate, heterozygous. This number has been selected so that our study will be 80% powered to detect a 40% change in allopurinol clearance.

GENDER OF SUBJECTS: Both males and females will be enrolled for this study. Because allopurinol has been shown to have detrimental effects on unborn fetuses, pregnant women will not be allowed to participate in the study.

AGE OF SUBJECTS: Healthy volunteers over 18 years of age will be enrolled

RACIAL AND ETHNIC ORIGIN: Subjects of Asian and European descent will be enrolled in this study. These ethnicities are known to carry the genetic variant of interest for this study.

INCLUSION CRITERIA:
1. Subject self-identify racial background; identify themselves, their parents, and their 4 grandparents as European or Asian;
2. Subjects are generally healthy with approved (normal) laboratory values for:
    - CBC Panel: WBC, RBC, Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, Platelet Count, MPV and Differential (Absolute and Percent - Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Basophils)
    - HFP: Total Protein, Albumin, Globulin (calculated), Albumin/Globulin Ratio (calculated), Total Bilirubin, Direct Bilirubin, Indirect Bilirubin (calculated), Alkaline Phosphatase, AST, ALT
    - RFP: Albumin, BUN/Creatinine Ratio (calculated), Calcium, Carbon Dioxide, Chloride, Creatinine, Estimated Glomerular Filtration Rate (calculated), Glucose, Phosphate (as Phosphorus), Potassium, Sodium, Urea Nitrogen
    - Uric Acid
3. Subjects with the ABCG2 genotype, homozygous, heterozygous or homozygous for the major allele of rs2231142 will be recruited;
4. Subjects >18 years of age and <40 years of age.

EXCLUSION CRITERIA:
1. Patients with vascular disease;
2. Patients with renal impairment (GFR < 60 ml/min);
3. Patients taking medications/supplements that affect uric acid levels;
4. Pregnant or lactating women;

5. Prior history of any allergic reaction to allopurinol/testing positive for HLA-B*5801 allele;
6. Risk of urinary or gastric retention or narrow-angle glaucoma (by medical history examination);
7. Impaired hepatic function (> 1.5 times the upper limit of normal);
8. Evidence of anemia (hemoglobin < 10 g);
9. Evidence or diagnosis of congestive heart failure;
10. Smokers;
11. Subjects with a mutation other than rs2231142 in the ABCG2 genotype;
12. Subjects taking hormonal contraceptives or other hormonal medications;
13. Evidence of recreational drug use as determined by questionnaire;

VULNERABLE SUBJECTS: Students may be enrolled in this study. This study poses little to no risks for healthy students. Only students over 18 will be enrolled and informed consent will be obtained.

METHODS AND PROCEDURES:

Questionnaire:

Effect of the Q141K BCRP variant on the pharmacokinetics and pharmacodynamics of Allopurinol – This questionnaire will be used to assess the subject's inclusion or exclusion from the study. The questionnaire will ask ancestry, health history, medications, habits, and family history. This questionnaire will help determine whether the subject is eligible to participate in the cheek swab portion of the screening.

Procedures, Timeline, and Biological Measures:

Screening Procedures: Part 1: Once eligible subjects have expressed their interest to participate and have had their questions answered by clinical personnel, the potential subject will be asked to email or call the research staff at Open Medicine Institute, Inc. If the eligible subject is already in the presence of the research staff they may proceed with the visit at the moment. During this visit the research staff will perform a screening evaluation for genotyping to determine eligibility. If email correspondence is preferred a secure network will be used for all correspondence between potential subjects and research staff.

Subjects will be asked to come to Open Medicine Institute, Inc in Mountain View, California, or other site determined by Kathleen M. Giacomini, Ph. D (P.I.), to complete a screening visit. The initial screening will include a review of the volunteer's medical history and history of drug use to identify any status that would determine eligibility. If the information on the health, drug use and ethnicity questionnaires are accurate, this will suffice for the inclusion criteria. Changes in medical or drug use history will be noted during the study. The purpose of these questionnaires is to characterize health status and ethnicity as accurately as possible (see Inclusion/Exclusion Criteria). If the volunteer is determined to be eligible to participate after the questionnaire, a cheek swab to determine

the subject's eligibility based on their ABCG2 genotype will be performed.

Part 2: If the genotype group of the subject is not yet full of enrolled subjects, the subject will then be asked to return to Open Medicine Institute, Inc. and an additional venipuncture of 10 ml will be drawn to screen for liver and renal insufficiency within 2 weeks of scheduled study visit. Subject's DNA will also be extracted (either from blood or additional cheek swab) to test for HLA*B 5801 allele, which is associated with allopurinol sensitivity. Female subjects' blood samples will be used for pregnancy testing (see Inclusion/Exclusion Criteria). Remaining saliva or blood sample may be stored for future genome-wide genotyping/sequencing.

The subject may not exercise, drink alcohol, or drink coffee. Strenuous exercise can affect uric acid excretion and alcohol and coffee, both caffeinated and decaffeinated, have been shown to affect uric acid levels.

Phase I procedures: Subjects will be asked to come to the clinic after 8 hours of fasting to provide a 10 ml blood sample to determine baseline serum uric acid and creatinine concentrations. Female subjects will be asked to provide a urine sample (20 mL) to ensure no change in pregnancy status. Subjects will be asked to empty their bladder and will then be given a placebo (microcrystalline cellulose). Blood samples will be collected at the following time points via an IV catheter: 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24 hours. Subjects will be asked to collect all urine samples during their stay. The volume of the urine will be recorded and 20 ml of the sample stored at -20°C for analysis of uric acid and creatinine content. The remaining urine will then be stored with all other urine samples from the subject until the last time point. Subjects will be asked to return in the morning for their 24 hour blood draw and final urine sample. Subjects will be asked to turn in any urine from overnight. 20 ml of the 24-hour collective urine sample will be stored at -20°C for analysis of uric acid and creatinine.

Phase II Procedures: Day 1: Subjects will be asked to come to the clinic after 8 hours of fasting to provide a 10 ml blood sample to determine baseline serum uric acid and creatinine concentrations. Female subjects will be asked to provide a urine sample (20 mL) to ensure no change in pregnancy status. Subjects will be asked to empty their bladders and will then be given 300 mg of allopurinol. Blood samples collected at the following time points via an IV catheter: 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24 hours. Subjects will be asked to collect all urine samples during their stay. The volume of the urine will be recorded and 20 ml of the sample stored at -20°C for analysis of uric acid, drug, and creatinine content. The remaining urine will then be stored with all other urine samples from the subject until the last time point. Subjects will be asked to return in the morning for their 24 hour blood draw and final urine sample. Subjects will be asked to turn in any urine from overnight. 20 ml of the 24-hour collective urine sample will be stored at -20°C for analysis of uric acid, drug, and creatinine.

Days 2-5: The subjects will receive 4 more doses of allopurinol to be taken once a day until their next visit. Their final inpatient visit must be on Day 6. Adherence will be measured via phone calls and patient diaries. Patients will be asked to keep a log of their

food and drinks.

Day 6: Subjects will be asked to return to the clinic after 8 hours of fasting for their final dose of allopurinol. Subjects will be asked to provide a 10 ml blood sample to determine steady-state levels of drug, creatinine, and uric acid. Subjects will be asked to empty their bladder and they will then be given 300 mg allopurinol. Following their final dose, blood and urine samples will be collected following Phase II: Day 1 procedures. Following the 24-hour time point, subjects will return at least two more times for blood and urine collection up to 72 hours post-allopurinol. **In total, subjects will provide approximately 400 ml of blood**. This is less than a typical blood donation of 500 ml and is below minimal risk of 550 ml for healthy subjects.

Visit	Timing	Collection
Screening Part 1	Anytime	Cheek Swab: ABCG2 genotype Questionnaire
Screening Part 2	Within two weeks of Placebo visit	Blood: CBC, HFP, RFP, UA If female - Pregnancy Cheek Swab: HLA*B 5801
Phase 1: Placebo	Before – Fast 8 hours clinic visit	Blood: Baseline- Creatinine, UA During at 0.5, 1,1.5, 2, 3, 4, 5, 6, 8, 10 hours (Creatinine, UA, ALLO/OXY) Urine: Before – females 20 ml for pregnancy test. During- all urine during stay collected
Phase 1: Placebo	24 hours after placebo dose clinic visit	Blood: Creatinine, UA, ALLO/OXY Urine: Turn in what was collected overnight
Phase 2: Allopurinol – Oral 300 mg dose	Before – Fast 8 hours Day 1—clinic visit *Must be started within 2 weeks of Day 0 (Placebo Phase)	Blood: Baseline- Creatinine, UA During at 0.5, 1,1.5, 2, 3, 4, 5, 6, 8, 10 hours (Creatinine, UA, ALLO/OXY_) Urine: Before – females 20 ml for pregnancy test. During- all urine during stay collected
Phase 2: Allopurinol – Oral 300 mg tablet	24 hours after first dose Day 2—clinic visit	Blood: Creatinine, UA, ALLO/OXY Urine: Turn in what was collected overnight – pregnancy test Patient given diaries and tablets to take home for Days 3-6
Phase 2: Allopurinol – Oral 300 mg tablet	48 hours after first dose Day 3 – at home	Patient Diary and phone follow-ups

Phase 2: Allopurinol – Oral 300 mg tablet	72 hours after first dose Day 4 – at home	Patient Diary and phone follow-ups
Phase 2: Allopurinol – Oral 300 mg tablet	96 hours after first dose Day 5 – at home	Patient Diary and phone follow-ups
Phase 2: Allopurinol Final Oral 300 mg tablet	120 hours after first dose Before – Fast 8 hours Day 6 – clinic visit	Blood: Baseline- Creatinine, UA, ALLO/OXY During at 0.5, 1,1.5, 2, 3, 4, 5, 6, 8, 10 hours (Creatinine, UA, ALLO/OXY) Urine: Before – females 20 ml for pregnancy test. During- all urine during stay collected
Phase 2: Allopurinol – Follow-up	24 hours after final dose Day 7 – clinic visit	Blood: Creatinine, UA, ALLO/OXY Urine: Turn in urine that was collected overnight
Phase 2: Allopurinol – Follow-up	Day 8 – clinic visit	Blood: Creatinine, UA, ALLO/OXY
Phase 2: Allopurinol – Follow-up	As close as possible to 72 hours after injection Day 9 – clinic visit	Blood:, Creatinine, UA, ALLO/OXY

ANALYTICAL METHODS: Measurement of allopurinol and oxypurinol will be performed by high performance liquid chromatography and mass spectrometry (LC-MS/MS). Measurement of uric acid and its precursors, xanthine and hypoxanthine, will be measured via Amplex Red and Abcam colorimetric assays.

Genotyping: All recruited subjects will sign informed consents and HIPAA forms. At screening, a blood sample (10 mL) or saliva sample will be obtained for DNA extraction. Plasma will be stored for assays of endogenous compounds and drugs. The ABCG2 variants will be genotyped at UCSF by a Taq Man Assay using primers and probes from ABI. The reaction will be run on an ABI 7900 HT.

HLA genotyping and wellness panels will be analyzed by qualified personnel at OMI or other reputable company as approved by Kathy Giacomini or other study personnel.

STATISTICAL ANALYSIS:

Mechanistic pharmacokinetic modeling will be conducted to characterize the pharmacokinetics of allopurinol (ALLO) and oxypurinol (OXY). The mechanistic model will characterize details relevant for this study such as conversion mechanism from ALLO to OXY and estimation of fraction of metabolized drug; estimation of ALLO and OXY renal and secretory clearance; possible concentration-dependent saturation

mechanism of secretory clearance and consequent longitudinal changes in OXY secretory CL and influence of pharmacogenomics (ABCG2) and demographic covariates (creatinine clearance, age, gender, race and body weight) on secretory and renal CL. By use of the physiological model, we will also attempt to estimate the drug concentration in the renal tubule; by knowing plasma and urinary concentration of OXY, and by estimating filtration and secretory clearance of OXY, we will be able to estimate the time profile of tubular concentrations of OXY. These tubular OXY concentrations represent concentration at the proposed site of action and they will further be linked with the pharmacodynamic response. A model describing concentration dependent inhibition of UA re-absorption from the renal tubule by OXY will be implemented. We will examine linear and non-linear (Emax type of model) relationships between tubular OXY and inhibition of UA reabsorption. In the pharmacokinetic modeling, we expect to see a significant influence of creatinine clearance on renal clearance and a significant influence of ABCG2 genotype on secretory CL and oral bioavailability, as described below. In the pharmacokinetic-pharmacodynamic analysis we expect that individuals with higher tubular concentrations of OXY will show the better response in terms of plasma UA lowering resulting in higher urine UA concentrations. Consequently, higher tubular concentrations will be mostly expected in individuals with high secretory clearance, e.g. individuals with ABCG2 141Q/Q, however the modeling may also account for other factors contributing to high tubular concentration, such as higher filtration rates and other factors, therefore providing more granularity and better mechanistic understanding in all processes leading to overall UA physiology. Although this specific PKPD analysis will be focused on the mechanism and role of ABCG2 in UA response to ALLO and OXY, the established mechanistic framework can be used to test the role of potential new pharmacogenetic variants. Longitudinal PKPD modeling using nonlinear mixed effects is a method carrying the most power to separate the signal from the noise; therefore modeling analysis will be associated with at least 80% power to detect differences in primary endpoints (net secretory clearance and change in UA) as described above. In addition to confirming the result of the primary analysis, this analysis will establish a mechanistic representation of the physiology underlying UA metabolism, therefore providing strong support and evidence to our mechanistic hypothesis. Pharmacokinetic parameters including renal clearance, and net reabsorptive or secretory clearance of UA, OXY and ALLO will be calculated from serum and plasma drug levels by standard pharmacokinetic methods. To assess net secretory or reabsorptive clearance of the three compounds, we will subtract fu*GFR (where fu is the unbound fraction of ALLO, OXY or UA and GFR is the glomerular filtration rate), as measured or estimated from serum and urine creatinine values, from renal clearance of ALLO, OXY and UA, directly measured from plasma and urine values. Previously we have used these methods to calculate net secretion (or reabsorption) of several drugs. For pharmacodynamics, differences in DSUA between the genotype groups will be evaluated first with an analysis of variance. If DSUA is not normally distributed, we will evaluate the differences between the genotype groups using a non-parametric Kruskal-Wallis test. We expect individuals with ABCG2 141K/K and 141Q/K will have a reduced response to ALLO (smaller DSUA) than individuals with ABCG2 141Q/Q. In addition, individuals with ABCG2 141K/K will have reduced renal clearance of ALLO and OXY in comparison to other genotypes since we propose that ABCG2 functions as a secretory

transporter for ALLO/OXY in the kidney. We will also determine whether the ABCG2 141K carriers have a reduced renal clearance.

DATA STORAGE AND CONFIDENTIALITY:
All personal and medical data will be considered confidential. Subjects will be assigned a unique number that will code for all personal data collected. This code will be used for all stored samples. All electronic study data will be stored on a password-protected computer on a secure network in the PI's laboratory, and backup files of the data will be stored on a hard disk which will be kept in a locked file cabinet or office.

Participation in research may involve a loss of privacy, but information about subjects will be handled as confidentially as possible and in compliance with HIPAA regulations. All information gathered in the study will be used collectively with information from other participants. All personal and medical data will be considered confidential. Upon the subjects enrollment in the study the researchers will assign them a number that will code for all of the personal data collected. Dr. Giacomini, her research associates, and team members will have access to information about each subject. Research staff, UCSF Committee on Human Research, and other University of California personnel may also review or receive information about the subject for quality assurance purposes (i.e. to review an adverse drug response). The subject's names or any identifying information will not be used in any published reports about this study, and data will be submitted and reported in consolidated, coded form. Furthermore, coded data obtained in the study will be deposited into the Pharmacogenetics Research Network Database (PharmGKB) on the World Wide Web. Researchers for further studies may access information on this database. However, the database will NOT include any information pertaining to the identity of the samples. Information identifying the subjects will not be available on the database.

For subjects enrolled at Open Medicine Insitute (OMI), access to patients' records will be limited to access by the study and clinic staff. OMI and site staff will go over the questionnaire with the volunteer to determine initial inclusion/exclusion. Each participating patient will be assigned a unique identification number. All data collected on each subject will be labeled with the corresponding study ID number. Patients will be enrolled in the study by providing signed written informed consent or by completing the existing web-based consent process system

Confidentiality of all records and materials will be maintained to the fullest extent possible. Written informed consent forms will be kept in locked files at participating sites or, if completed online, password protected.

All data generated by OMI will be stored on OMI's state of the art data center in Fremont, CA. This center runs a VMware distributed architecture with a large EMC2 VNX data store with their own proprietary OpenMedNet layer. This ensures data integrity and security.

RISK CATEGORY: Minimal

POTENTIAL RISKS:

1. Allopurinol: Allopurinol is a xanthine oxidase inhibitor used to treat gout and hyperuricemia that can be dosed from 100 mg to 800 mg daily. The most frequent side effects are skin rash (1.5%), diarrhea (1.3%), nausea (1.2%), and acute gout attacks. Furthermore, these side effects occur in less than 1% of those patients dosed and at a dose of 300 mg/day, we do not expect to see any discomfort in the volunteers.
    a. **Common but less serious:**
        • Diarrhea
        • Drowsiness
        • Headache
        • Change in taste
        • Muscle pain
    b. **Rare but serious**
        • Renal Failure
        • Stevens-Johnson Syndrome*

* Although some patients develop Stevens-Johnson syndrome on allopurinol, subject's side effects will be monitored closely and dosing will be discontinued with any signs of skin rash.

2. Blood loss and Venipuncture: Withdrawal of blood from a vein may cause pain, bruising, and in rare cases infection at the site of needle puncture. Some people may faint when blood is drawn. All subjects will provide approximately 400 mL of blood. Loss of 400 mL of blood is not harmful and will be replenished by the body within 2-3 weeks.

3. Questionnaire: Some of the questions may make the participants feel uncomfortable. All participants will be informed that they have the right to decline to answer any questions.

4. Genetic Testing: Participation in this study may involve a loss of privacy. All DNA testing will be performed using blood/saliva samples tagged with a unique identification number, to minimize the risk of loss of confidentiality. Only the research staff will have access to the samples and records and the key to the code. Information from this study used for scientific publication will not contain any identifying information. Subjects may withdraw consent for DNA storage at any time during or after the study by writing a letter to REDACTED
5. Inconvenience of Study Visit: Subject may experience an inconvenience by having to attend multiple study visits while study procedures and questionnaire data are being collected.

PROTECTION AGAINST RISKS:
To minimize the risks/discomforts to subjects, subjects will be excluded from

participation if:
1. Under 18 years old
2. Patients with vascular disease
3. Patients with renal impairment (GFR < 60 ml/min)
4. Patients taking medications that affect uric acid levels
5. Pregnant or lactating women
6. Prior history of any allergic reaction to allopurinol
7. Risk of urinary or gastric retention or narrow-angle glaucoma (by medical history examination)
8. Impaired hepatic function (> 1.5 times the upper limit of normal)
9. Evidence of anemia (hemoglobin < 10 g)
10. Evidence or diagnosis of congestive heart failure
11. Evidence of recreational drug use

To further minimize risks, OMI is equipped with emergency medical tools such as ACLS trained personnel, crash carts, and emergency drugs and supplies to stabilize any subject until emergency personnel arrive after 911 is called. El Camino Hospital is directly across the street in case of emergencies as well.

BENEFITS TO SUBJECTS:
The benefit of participating in this studies is that you are able to add to our knowledge about how the pharmacokinetic and pharmacodynamic mechanisms by which Q141K (rs2231142) associates with uric acid lowering in gout patients on allopurinol with relatively little inconvenience to you. While these types of studies are unlikely to offer direct benefit to your disease at the time of your involvement, the information gained may be used to develop new therapies to improve survival or quality of life for you or other patients like you in the future.

ALTERNATIVES TO PARTICIPATION:
The subject can choose not to participate in this study.

SUBJECT IDENTIFICATION AND RECRUITMENT:

Subjects will be recruited from Asian and European populations within reasonable driving distance of Open Medicine Institution (OMI), with the initial focus on Asian populations. OMI will also reach out to locations where Asian and European descent populations are prevalent to request permission to hand out or hang an IRB approved flyer. We will also post a booth with the flyer at other local sites. Interested individuals may contact OMI directly through the information on the flyer to begin the screening process. If research personnel are available in person the interested individual may also talk directly to the research personnel at that time in a private location.

PROCESS OF CONSENT:

At OMI, all research intervention will be conducted in a private room to maintain privacy. All subjects are required to sign an Informed Consent Form prior to participation

in compliance with 21CFR50 Subpart B. Open Medicine Institute (OMI) will receive approval of the Informed Consent Form from the Western Institutional Review Board (WIRB) as well as continuing review approval, as necessary, in compliance with 21CFR812 Subpart D.

All subjects must be provided a copy of the Informed Consent and the California Experimental Bill of Rights but the bill of rights and consent may be done either using the electronic informed consent on https://www.OpenMedNet.org or using written informed consent. If the subject signs the electronic consent form this form is always available for view by the subject, or the subject may print it out, so a signed written copy of the informed consent is not necessary. If the subject signs a written copy the subject must receive a copy of the signed and dated consent document. The original copy of the consent form will be retained at OMI in a secure, locked location. Each subject may be enrolled into the study only once.

Subjects must be explained the purpose of the study in terms understandable to them with sufficient information for them to make an informed decision regarding their participation. They must be informed and understand that participation is always voluntary and that participation in the study will not impact the clinical care that they currently receive. The consent form will specify whom to contact if there are injuries as a result of the study and whom to contact with general questions about the study. The consent form will notify subjects that blood, urine and DNA samples will be collected and will be used for research purposes and stored for the purpose of conducting future research by UCSF, including genetic DNA sequencing.

SUBJECT CAPACITY:
Subjects will only be enrolled if they are able to give informed consent themselves.

SUBJECT COMPREHENSION:
Informed consent will be obtained using the form attached to this protocol. Subjects will be briefed on all procedures in the trial in plain speech and will have the opportunity to have all of their questions answered. Subjects will indicate that they understand the risks and benefits and agree to participate in the study by signing the informed consent form.

DOCUMENTATION OF CONSENT:
Signed consent forms will be retained on a secure server along with the subject information as previously described.

COSTS TO THE SUBJECT:
There will be no cost to the subject other than the time it takes to participate in this study (approximately 36.5 hours).

PAYMENT FOR PARTICIPATION:
If subjects agree to be screened, they will receive a $10 payment for the initial screening visit (cheek swab). If they qualify and decide to continue onto the secondary screening, they will receive an additional $15. If they are enrolled into the remaining phases of the

study, they will be eligible to receive an additional $575 payment to offset their time and inconvenience. Only if they complete everything and comply with all aspects of the study will they be paid the entire amount of $575 (in addition to the $25 received for the screening for a total of $600). If they fail to comply any portion of the study visit or do not complete the entire visit then they will only receive a prorated payment based on the time of participation (approximately $10.0 /per hour). Payments will be paid in the form of a check or debit card at the completion of the study, which they should receive four to six weeks after the last visit. Screening visit may be paid in cash or check. Subjects must provide the researchers their Social Security number and mailing address so the check or debit card can be processed. Transportation and Parking fees may be provided if requested in advance. According to law, subjects will have to pay taxes on payment for research participation in excess of $600 per calendar year.

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APPENDICES:

Appendix 1. Allopurinol Informed Consent Form

Appendix 2. Questionnaire: Effect of the Q141K BCRP variant on the pharmacokinetics and pharmacodynamics of allopurinol.

Appendix 3. Subject Diary

Appendix 4. Allopurinol Subject Recruitment Flyer

Appendix 5. Allopuinol Insert

Apendix 6. Allopurinol AGC-9588