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Evaluation of polymorphisms in the sulfonamide detoxification genes *NAT2*, *CYB5A*, and *CYB5R3* in patients with sulfonamide hypersensitivity

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Abstract

Objective—To determine whether polymorphisms in the sulfonamide detoxification genes, *CYB5A* (encoding cytochrome *b*₅), *CYB5R3* (encoding cytochrome *b*₅ reductase), or *NAT2* (encoding *N*-acetyltransferase 2) were over-represented in patients with delayed sulfonamide drug hypersensitivity, compared to control patients that tolerated a therapeutic course of trimethoprim-sulfamethoxazole without adverse event.

Methods—DNA from 99 non-immunocompromised patients with sulfonamide hypersensitivity that were identified from the Personalized Medicine Research Project at the Marshfield Clinic, and from 99 age-, race-, and gender-matched drug-tolerant controls, were genotyped for four *CYB5A* and five *CYB5R3* polymorphisms, and for all coding *NAT2* SNPs.

Results—*CYB5A* and *CYB5R3* SNPs were found at low allele frequencies (less than 3–4%), which did not differ between hypersensitive and tolerant patients. *NAT2* allele and haplotype frequencies, as well as inferred *NAT2* phenotypes, also did not differ between groups (60% vs. 59% slow acetylators). Finally, no difference in *NAT2* status was found in a subset of patients with more severe hypersensitivity signs (drug reaction with eosinophilia and systemic symptoms; DRESS) compared to tolerant patients.

Conclusions—We found no evidence for a substantial involvement of these 9 *CYB5A* or *CYB5R3* polymorphisms in sulfonamide HS risk, although minor effects cannot be completely ruled out. Despite careful medical record review and full re-sequencing of the *NAT2* coding region, we found no association of *NAT2* coding alleles with sulfonamide hypersensitivity (predominantly cutaneous eruptions) in this adult Caucasian population.

Keywords

sulfamethoxazole; potentiated sulfonamides; drug hypersensitivity; *N*-acetyltransferase; cytochrome *b*₅; hydroxylamine

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Introduction

Potentiated sulfonamide antibiotics, such as sulfamethoxazole in combination with trimethoprim (TMP-SMX), are a common cause of idiosyncratic delayed drug hypersensitivity reactions. ^[1,2] Skin rash and fever, occurring five or more days after drug initiation, are the most frequent manifestations, although hepatopathy, blood dyscrasias, and involvement of other organ systems have also been reported. ^[3] Notably, TMP-SMX is the leading cause of Stevens-Johnson syndrome and toxic epidermal necrolysis, associated with 30% mortality. ^[4]

SMX hypersensitivity affects about 3% of the general population, ^[3] with an apparent familial predisposition in non-immunocompromised patients. ^[5–7] This suggests a genetic component to these adverse drug reactions. SMX hypersensitivity has been attributed to the generation of a hydroxylamine metabolite (SMX-HA). ^[8–10] SMX-HA spontaneously oxidizes to its unstable nitroso derivative (SMX-NO), which forms tissue haptens; ^[11,12] these haptens stimulate the production of anti-SMX antibodies and drug-specific cytotoxic T cells. ^[12–15] SMX-HA is generated by CYP2C9 in the liver; ^[16] however, high activity variants of this enzyme, which might enhance the risk of SMX hypersensitivity, have not been identified. ^[17]

One detoxification pathway for sulfonamides is *N*-acetylation of the parent drug by *N*-acetyltransferases (NATs), leading to an inactive metabolite that is eliminated in the urine. Polymorphisms in the *NAT2* gene that lead to a defective “slow” *N*-acetylation phenotype are well described, and slow NAT2 phenotype and genotypes have been reported to be overrepresented in patients with SMX hypersensitivity. ^[18–22] However, since slow *NAT2* polymorphisms are found in about half of Caucasians and African Americans, these genotypes alone are not sufficient to lead to SMX hypersensitivity in most patients. ^[23]

Another detoxification pathway for sulfonamides that we have recently characterized is cytochrome *b*₅ (b5) and NADH cytochrome *b*₅ reductase (b5R). These enzymes reduce SMX-HA back to the non-reactive parent SMX, ^[24] via a detoxification pathway that is 10 times more efficient than forward oxidation to SMX-HA. ^[16] SMX-HA reduction by b5 and b5R varies more than 19-fold in human liver, and we have identified multiple promoter, coding, and 3' UTR polymorphisms in the genes encoding b5 (*CYB5A*) and b5R (*CYB5R3*) in subjects with low to undetectable hepatic SMX-HA reduction activities. ^[25] Thus, defective polymorphisms in either *CYB5A* or *CYB5R3* could lead to increased availability of SMX-HA for eventual hapten formation, and could contribute, along with slow *NAT2* alleles, to the individual risk of sulfonamide hypersensitivity.

The primary aim of this study, therefore, was to determine whether polymorphisms in *CYB5A* or *CYB5R3*, along with slow *NAT2* genotypes, were over-represented in non-immunocompromised patients with sulfonamide hypersensitivity, compared to patients who tolerated a prescribed therapeutic course of TMP-SMX.

Methods

Patient samples

Medical records of patients enrolled in the Personalized Medicine Research Project (PMRP), ^[26] a cohort of over 20,000 patients who receive their medical care at the Marshfield Clinic, Marshfield, WI, were searched electronically for a history of TMP-SMX administration or for a diagnosis of sulfonamide hypersensitivity. These patients have previously donated blood samples for a genomic DNA bank, and provided informed consent for the use of their samples for biomedical research, linked to de-identified medical records

data. Marshfield Clinic Research Foundation staff individually reviewed medical records, using a structured abstraction form to identify patients with sulfonamide hypersensitivity (HS). Each case was adjudicated to ensure consistency and accuracy. The abstraction form included the following eligibility criteria: 1) administration of TMP-SMX for at least 5 days prior to the adverse event;^[3] 2) documentation of one or more new clinical signs after starting TMP-SMX, including fever with or without eosinophilia, skin rash, increases in liver enzyme activities, hyperbilirubinemia, blood dyscrasias, pneumonitis, myocarditis, aseptic meningitis, polyarthrititis, acute interstitial nephritis, toxic epidermal necrolysis, or Stevens-Johnson syndrome;^[3] 3) lack of other clinical explanation for the adverse event; 4) absence of other drugs being administered that are known to cause rash in patients; and 5) resolution of clinical signs with discontinuation of TMP-SMX and no other interventions or changes in other drug regimens. Patients with only gastrointestinal symptoms such as nausea, vomiting or diarrhea,^[3] or with acute anaphylactoid reactions,^[27,28] were excluded. Because some forms of immunosuppression, in particular AIDS, lead to a high acquired risk of SMX hypersensitivity, possibly independent of genotype,^[17] immunocompromised patients, including those with HIV infection or undergoing immunosuppressive therapy, were not eligible. These criteria together in the abstraction form were designed to yield a score of 6 or more, or “probable” adverse reaction, using the Naranjo Adverse Drug Reaction scale.^[29] Data from any records with questionable abstraction criteria were reviewed by two authors (LT and SY) to reach consensus; records with vague or conflicting information were eliminated from eligibility.

Control patients (“tolerant;” TOL) within PMRP that were prescribed TMP-SMX were enrolled sequentially from medical records in random order, to provide a comparable group to the HS patients based on race, gender, and decade of age when TMP-SMX was prescribed. Control patients must have been prescribed a course of TMP-SMX at a standard therapeutic daily dosage for at least 10 days, with adequate follow-up in the medical record to indicate that the drug was taken and tolerated without adverse event. Clinical and demographic variables, including age at dosing, gender, race, body weight, dosage, duration of treatment, and reason for TMP-SMX prescription (respiratory, urinary tract, or other soft tissue infection) were also abstracted. A sample size calculation, using a Chi-Square distribution and an $\alpha = 0.05$, indicated that 100 HS cases and 100 TOL controls would provide at least 80% power to detect an odds ratio of 2.33 or higher for common alleles (minor allele frequency of 0.25), and 4.01 or higher for less frequent alleles (minor allele frequency of 0.04).

Genotyping for *CYB5A* and *CYB5R3* polymorphisms

Samples were genotyped for four *CYB5A* single nucleotide polymorphisms (SNPs; c.13T>G, c.178A>G, -389G>A, and -382C>T), and five *CYB5R3* SNPs (c.890G>A, I1+6 C>T, -251G>T, *392G>C, and *863T>C). These SNPs were previously identified in re-sequencing of the promoter, coding, and 3' untranslated regions of *CYB5A* and *CYB5R3* in more than 180 subjects,^[25,30,31] and were observed in association with decreased protein expression and/or low hepatic SMX-HA reduction activities in individual subjects.^[25,30] The PCR-based Taqman Genotyping Assay (Applied Biosystems, Foster City, CA), through the University of Wisconsin Biotechnology Center, was utilized for most polymorphisms. SNPs that failed the Taqman assay were evaluated by pyrosequencing, using the PSQTM96MA System (Biotage AB, Uppsala, Sweden). Both SNP screening techniques were validated by running positive and negative genomic DNA controls from liver or breast samples, in which the allele of interest had been previously established by direct sequencing.^[25,32]

Resequencing for *NAT2* polymorphisms

NAT2 is a small gene with an intronless coding region,^[33] for which multiple SNPs have been identified in association with low activity (<http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature/>). Therefore, the entire *NAT2* coding region was resequenced for each subject, using primers designed to the 5' and 3' flanking regions of the open reading frame in exon 2 (F: CATGTAAAAGGGATTTCATGCAG; R: CGTGAGGGTAGAGAGGATATCTG, encompassing 5' 11 –77 to 3' *90). *NAT2* coding sequences were analyzed for polymorphisms using open source DNA analysis software (Staden Package; <http://staden.sourceforge.net/>). All identified coding polymorphisms were confirmed by direct review of sequencing chromatograms, and by re-amplification and re-sequencing to rule out experimental artifacts.

Statistical analyses

Clinical and demographic variables, to include age at dosing, sex, race, body weight, dosage, duration of treatment, and reason for TMP-SMX prescription (respiratory, urinary tract, or other soft tissue infection) were compared between groups using a Mann Whitney U test or chi-square statistic, as appropriate. Prior to association analysis, genotypes were screened using quality control filters; SNPs were checked for genotyping efficiency (such that only genotypes or individuals with missing data rates less than 5% were included) and tested for deviation from genotype proportions expected under Hardy-Weinberg equilibrium (HWE) conditions using Fisher's exact tests. Allele frequencies for *CYP5A*, *CYP5R3*, and *NAT2* variants in HS patients were compared to those in control patients using Chi square or Fisher's exact tests, depending on the expected number of observations in the contingency table cells; a Bonferroni correction for the number of tests was used to correct with multiple comparisons (the alpha level cut-off for significance was adjusted based on the number of tests performed). In addition, interactions among genotypes in relation to SMX hypersensitivity risk were evaluated using Multifactor Dimensionality Reduction, a commonly used data-mining approach designed to detect gene-gene and gene-environment interactions.^[34,35] The quality control and univariate analyses were performed in Stata v11, and the MDR analysis was performed in R, using the MDR.R package.^[36]

For *NAT2*, haplotypes were assigned using standard *NAT2* haplotype nomenclature (<http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature/>). A Fisher's exact test was then performed to test for association between *NAT2* haplotype and HS status; rare haplotypes with less than 5 observations in the whole dataset were collapsed into a single category for analyses. Finally, individual patients were assigned a *NAT2* phenotype using a public web server that utilizes pattern recognition to infer phenotype from genotypes at each of 6 polymorphic *NAT2* coding sites (282, 341, 481, 590, 803, and 857; nat2pred.rit.albany.edu).^[37] Prevalence of inferred phenotypes (fast, intermediate, or slow acetylator) were then compared between HS and TOL patients using Fisher's exact tests.

Results

Patient demographics

Ninety-nine HS patients were available for study. The most common documented clinical signs of HS were rash, fever, and eosinophilia, which were noted a median of 8.0 days after starting TMP-SMX (Table 1). Sixteen patients (16%) had adequate data reported to meet the criteria for DRESS (drug reaction with eosinophilia and systemic symptoms), defined as 3 or more of the following: acute rash, fever, lymphadenopathy, involvement of an internal organ, and blood count abnormalities (lymphopenia or lymphocytosis, eosinophilia, or

thrombocytopenia).^[38] Only one HS patient was re-challenged with TMP-SMX, and this patient also developed skin rash with re-exposure. Most medical records did not have an adequate family history regarding sulfonamide hypersensitivity; of 19 patients queried, one reported both a sister and a mother affected.

Ninety-nine control (TOL) patients were also enrolled. Thirty of these tolerant patients were prescribed an additional second course of TMP-SMX without adverse event. Dosage of TMP-SMX, body weight, and median duration of prescription (10 days) were not different between groups (Table 1). However, significantly fewer HS patients were prescribed TMP-SMX for soft tissue infections (2%) compared to tolerant patients (15%; $P = 0.0015$).

There were no significant differences between TOL and HS patients with regard to age at treatment, race, or sex, as these were controlled for in our matching protocol. Eighty-two of the HS patients were female (82.8%), which was significantly higher than the 69.3% female prevalence in the larger PMRP database of patients that were prescribed TMP-SMX without recorded adverse event ($n = 939$; $P = 0.017$).

CYB5A and CYB5R3 genotyping

No genotypes or individuals were removed based on quality control checks (no missing data for more than 5% of subjects, and all P values from HWE tests > 0.05). All of the 4 *CYB5A* SNPs were found at low allele frequencies (less than 4% of patients in both groups), and none were found at significantly different frequencies between HS and TOL patients (Table 2; $P = 0.17$). The 5 *CYB5R3* SNPs were also found at low allele frequencies (less than 3% of patients in both groups), and were also not different between groups (Table 2; $P = 0.37$).

NAT2 re-sequencing

Eight SNP's were identified during resequencing of the *NAT2* coding exon across all individuals. A novel SNP, *NAT2* 246C>T, was found in only one individual in the heterozygous state. The other seven SNPs were found with allele frequencies ranging from 0.005 to 0.747 (Table 3). However, there were no significant differences in minor allele, genotype, or haplotype frequencies between HS and TOL patients (Bonferroni adjusted rejection criteria = 0.007). Further, there were no detectable interactions among *CYB5A*, *CYB5R3*, and *NAT2* genotypes using Multifactor Dimensionality Reduction testing (largest accuracy for any model was 55.05%, $P < 0.71$).

Individual patients were assigned a *NAT2* phenotype using a public web server that utilizes pattern recognition to infer *NAT2* phenotype from genotypes at each of 6 polymorphic coding sites (282, 341, 481, 590, 803, and 857; nat2pred.rit.albany.edu).^[37] A majority of HS patients were predicted to be slow acetylators (60%, 59 of 99), which was not different from TOL patients (59%, 58 of 99). When patients with DRESS were analyzed separately, there were still no differences in predicted *NAT2* phenotypes between these more severely affected patients (63% slow acetylators) and TOL patients (59%). A post hoc power calculation for this subset of patients (using the same assumptions as described for the primary outcome power calculations) indicated that the current study had 80% power to detect odds ratios of 4.26 or higher for common *NAT2* alleles (MAF > 0.25) in patients with DRESS, but only 7.18 or higher for lower frequency variants (MAF < 0.04).

Discussion

We hypothesized that defective SNPs in *CYB5A* and *CYB5R3*, along with slow *NAT2* genotypes, would be associated with an increased risk of SMX drug hypersensitivity. We addressed this hypothesis by retrospectively identifying patients with a diagnosis of SMX hypersensitivity through the Personalized Medicine Research Project at Marshfield Clinic,

and by comparing allele frequencies between HS and TOL patients for 9 *CYB5A* and *CYB5R3* polymorphisms, previously observed in association with low individual SMX-HA reduction and/or protein expression in genotype-phenotype surveys. [25,30] In addition, we re-sequenced the *NAT2* coding region and compared allele, genotype, and haplotype frequencies between HS and TOL patients, as well as potential interactions among *CYB5A*, *CYB5R3*, and *NAT2* genotypes and HS outcome.

Most of the HS patients were female (83%), which was significantly higher than the percentage of patients in the PMRP database that were prescribed TMP-SMX without adverse event (69% female). A prospective study of more than 1,100 TMP-SMX prescriptions reported that 11% of women, but only 5% of men, developed skin rashes, [39] and a recent Thai study of cutaneous reactions to sulfonamide antibiotics reported a female:male ratio of 1.5 to 1; however, this latter result was not adjusted for the number of prescriptions made by gender. [40] Conversely, a smaller prospective study of 359 SMX prescriptions found no gender difference in the incidence of “allergic” reactions, although specific diagnostic criteria were not given, [41] and a retrospective study of 969 sulfonamide HS patients and 19,257 tolerant controls found no gender differences, although diagnostic codes for both acute and delayed HS were combined in the analysis. [42] Therefore, it is unclear whether our findings represent a true gender predisposition for delayed SMX HS in this population, or result from unidentified co-morbidities or reporting bias.

We found that most *CYB5A* and *CYB5R3* polymorphisms were uncommon in the control population of TOL patients. These findings are consistent with our previous observations in histologically normal human liver and breast tissue, in which non-synonymous polymorphisms in either gene were found at relatively low allele frequencies in healthy Caucasians. [25,31] In the present study, we further found a similarly low prevalence of *CYB5A* and *CYB5R3* polymorphisms in patients with sulfonamide HS; in addition, all *CYB5A* and *CYB5R3* variants, when observed, were in the heterozygous state. This may reflect evolutionary pressure to conserve the function of this pathway, which has an important endogenous role in maintaining hemoglobin in its functional, reduced state. [43–45] We did not fully re-sequence both genes in the HS and TOL patients, however, so we may have missed one or more important polymorphisms that were unique to HS patients. In addition, our matching protocol did not allow us to detect any interactions between sex or age at the time of adverse event, with any of the genotypes. Our sample size was able to detect odds ratios of 2.33 or higher for common alleles, and 4.01 or higher for low allele frequencies (i.e. MAFs of 0.04), although many of our *CYB5A* and *CYB5R3* MAFs were even lower than this threshold. Based on the allele with the largest apparent divergence in MAFs between groups (–382C>T; MAFs of 0.035 in HS and 0.010 in TOL), we would have needed 530 patients with sulfonamide HS and 530 tolerant controls to show this to be a significant risk factor, with 80% power. However, given the low MAFs even in the affected patients, this and the other *CYB5A* and *CYB5R3* alleles that we tested are unlikely to have even a modest impact on sulfonamide HS outcome in the general (Caucasian) population.

NAT2 polymorphisms were found in TOL control patients with allele frequencies that were similar to those previously reported in Caucasians, [23,46,47] and there were no significant differences in allele or genotype frequencies between TOL and HS patients. Further, when our patients were assigned an inferred *NAT2* phenotype, the proportion of HS patients with slow acetylator status (60%) was not significantly different from TOL patients (59%). This is in contrast to two earlier small phenotyping studies using caffeine as a probe, which showed a high proportion of HS patients with the slow acetylator phenotype (90–100%). [6,18] Most of these patients were children, and phenotype prevalence was compared to adult control populations (~55% slow acetylator prevalence). [6,18] Young children may have discordant acetylation phenotype and genotype due to differences in caffeine

disposition, and a later study showed that 23% of children with wild type *NAT2* genotypes were falsely assigned to slow acetylator status based on caffeine administration.^[48] Therefore, age-related discordance between acetylator genotype and phenotype status could explain, in part, the differences between our findings and previous phenotyping studies.

Other studies have looked directly at *NAT2* genotype and sulfonamide HS (Table 4). No associations with sulfonamide HS were found for *NAT2* alleles in two studies of HIV-infected patients,^[17,49] which is consistent with the hypothesis that the acquired high risk of SMX HS in this population may involve environmental factors.^[50] In non-immunocompromised patients, the synonymous *NAT2* SNP 481C>T was found in 14 of 18 patients (78%) with severe bullous skin eruptions from sulfonamide antibiotics,^[20] and with a higher allele frequency in 14 infants with sulfonamide HS compared to 7 drug-tolerant babies.^[22] A later study in 29 children with TMP-SMX HS found a significantly higher prevalence of slow 590 G>A and 857 G>A alleles, compared to 19 drug-tolerant patients, and no HS patients had a wild type *NAT2* genotype.^[21] Finally, in Japanese patients with systemic lupus erythematosus, the wild type *NAT2**4 allele was significantly less common in 18 patients that developed drug HS compared to patients that tolerated TMP-SMX.^[19] In all of these *NAT2* genotyping studies, the number of HS patients was relatively small, and a relatively high proportion of patients had systemic signs other than simple rash, to include hepatopathy, blood dyscrasias, and bullous skin eruptions (69–100% of affected patients).^[19–22] Most of the patients in our study had SMX HS that was limited to cutaneous involvement, and we could have missed *NAT2* genotype associations in the subset of patients with more severe systemic manifestations of sulfonamide HS.

In addition, there is always a concern that some HS patients in our study, as well as in previous studies without complete medical record review, may have developed cutaneous eruptions due to other, unidentified causes, and were therefore misclassified. Our study design took advantage of the Personalized Medicine Research Project (PMRP) at the Marshfield Clinic, Marshfield, WI, for which DNA samples have been banked, with informed consent, from more than 20,000 patients under Marshfield Clinic care. These samples are linked to complete medical records that follow patients for, in some cases, decades. However, most patients were not re-challenged with TMP-SMX to confirm the diagnosis of sulfonamide hypersensitivity. Although this is considered the gold standard for confirmation of an idiosyncratic reaction, it is typically not pursued because of risk to the patient. In order to enhance the quality of our study population, we performed complete chart reviews rather than relying on diagnostic codes, and adjudicated all cases. This allowed us to categorize patients using the Naranjo Adverse Drug Reaction scale prior to inclusion,^[29] including only patients with a “probable” or higher score, in an attempt to minimize misclassification.

Overall, our data do not support an association between these candidate *CYB5A* and *CYB5R3* SNPs, or *NAT2* coding SNPs, with sulfonamide HS in this population of Caucasian patients with primarily cutaneous eruptions. While our study was adequately powered for moderate effects, we had limited power to detect very small effect sizes, especially for rare variants, and we cannot rule out minor contributions of these alleles. In addition, evaluation of larger group of patients with more severe manifestations of SMX HS is indicated. Follow-up studies are planned to further examine genetic risk of SMX HS from a broader perspective, using genome wide association techniques in an expanded group of patients from the PMRP and other populations.

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Table 1

Patient demographics for sulfonamide hypersensitive and sulfonamide tolerant patients enrolled from the Marshfield Clinic Personalized Medicine Research Project.

	Hypersensitive (n = 99)	Tolerant (n = 99)
Median age at dosing ¹	38.5 years (range, 1.1 to 81.6 years)	39.3 years (range, 8.5 to 87.7 years)
Gender ¹	82 females 17 males	82 females 17 males
Race ¹	96 Caucasians 3 Native Americans	97 Caucasians 1 Native American 1 Race not specified
Median body weight	80 kg (range, 13 to 124 kg)	77 kg (range, 27 to 150 kg)
Reason for prescription	Respiratory infection (62%) Urinary tract infection (36%) Other soft tissue infection (2%)	Respiratory infection (60%) Urinary tract infection (25%) Other soft tissue infection (15%)
Median dosage of TMP-SMX	24.1 mg/kg/day (range, 10.3 to 37.5 mg/kg/day)	25.0 mg/kg/day (range, 13.0 to 71.0 mg/kg/day)
Median duration of prescription	10 days (range, 7 to 42 days)	10 days (range, 10 to 30 days)
Median time to adverse event	8.0 days (range, 5 to 21 days)	NA
Adverse event (% patients affected)	Rash (94.9%) Fever (18.2%) DRESS ² (16.2%) Eosinophilia (15.2%) Thrombocytopenia (9.1%) Neutropenia (5.1%) Anemia (4.4%) Increases in liver enzymes or bilirubin (2.0%) Stevens-Johnson syndrome (1.0%).	NA

¹Tolerant patients were matched to hypersensitive patients by gender, race, and age at dosing

²Drug reaction with eosinophilia and systemic symptoms, defined as 3 or more of the following: acute rash, fever, lymphadenopathy, involvement of an internal organ, and blood count abnormalities (lymphopenia or lymphocytosis, eosinophilia, or thrombocytopenia). [38]

Minor allele frequencies for 4 *CYB5A* and 5 *CYB5R3* SNPs, genotyped in 99 patients with sulfonamide hypersensitivity (HS) and 99 sulfonamide-tolerant patients (TOL). These SNPs were previously observed in individual human livers in association with low sulfamethoxazole hydroxylamine reduction, and low b5 or b5R protein expression (non-coding SNP data unpublished). [25] There were no significant differences in minor allele frequencies between hypersensitive and tolerant patients. Odds ratios (OR) and confidence intervals (CI) are provided which compare groups with the variant allele versus the reference allele.

Table 2

SNP	Location	Amino acid change	Ref SNP ID	Minor allele frequency		OR (95% CI)
				HS	TOL	
CYB5A						
c.13T>G	Exon 1	Ser5Ala	rs75160992	0.005	0.005	1.00 (0.06–16.2)
c.178A>G	Exon 2	Thr60Ala	rs78009726	0.000	0.000	NA
–389G>A	Promoter	-	rs77005399	0.000	0.005	NA
–382C>T	Promoter	-	rs76631379	0.035	0.010	4.13 (0.45–37.6)
CYB5R3						
c.890G>A	Exon 9	Arg297His	rs76458556	0.015	0.005	2.02 (0.18–22.7)
I1+6 C>T	Intron 1	-	rs8190370	0.029	0.018	1.52 (0.25–9.3)
–251G>T	Promoter	-	rs73888347	0.000	0.000	NA
*392G>C	3' UTR	-	rs7284807	0.005	0.015	0.49 (0.04–5.6)
*863T>C	3' UTR	-	rs77499608	0.005	0.005	1.00 (0.06–16.2)

NA = Odds ratio not applicable (numerator or denominator is zero).

Frequencies for *NAT2* alleles, found via genomic DNA re-sequencing of the *NAT2* coding exon in 99 patients with sulfonamide hypersensitivity (HS) and 99 sulfonamide-tolerant (TOL) patients. Odds ratios (OR) and confidence intervals (CI) are provided to compare groups with the variant allele versus the reference allele.

Table 3

Variant	Amino acid change	Ref SNP ID	Minor allele frequency		OR (95% CI); <i>P</i> value ^f
			HS	TOL	
246 C>T	Synon	Novel	0.0005	0.000	NA <i>P</i> = 1.000
282 C>T	Synon.	rs1041983	0.288	0.323	0.87 (0.47–1.58) <i>P</i> = 0.70
341 T>C ²	Ile114Thr	rs1801280	0.495	0.460	1.13 (0.65–1.97) <i>P</i> = 0.15
481 C>T	None	rs1799929	0.455	0.399	1.28 (0.73–2.25) <i>P</i> = 0.41
590 G>A ²	Arg197Gln	rs1799930	0.252	0.308	0.74 (0.40–1.38) <i>P</i> = 0.46
803 A>G ³	Lys268Arg	rs1208	0.495	0.414	1.39 (0.79–2.43) <i>P</i> = 0.018
857 G>A ²	Gly286Glu	rs1799931	0.025	0.010	3.06 (0.31–29.9) <i>P</i> = 0.248

^f Bonferroni adjusted rejection criteria = 0.007.

² Variants that are predicted to have a slow *NAT2* phenotype in the homozygous state

³ Variant predicted to have a rapid *NAT2* phenotype in the homozygous state (<http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature/>).

NA = Odds ratio not applicable (numerator or denominator is zero).

Table 4

Summary of previous published results for *NAT2* genotyping in patients with sulfonamide hypersensitivity (HS), versus patients tolerant of TMP-SMX (TOL). Minor allele frequencies in bold were reported to be significantly different between groups.

Population	Sulfonamide HS manifestations	Genotyping method	Minor allele frequency ¹		Publication
			HS	TOL	
Immuno-competent adult patients; French	Toxic epidermal necrolysis or Stevens-Johnson syndrome	RFLP ²	n = 18		Wolkenstein, 1995 [20]
			481T	0.528	
			590A	0.361	
			857A	0.028	
Infants suspected of Pneumocystis; Polish	Blood dyscrasias hepatopathy, skin lesions	RFLP	n = 13	n = 7	Zielinska, 1998 [22]
			481T	0.615	
			590A	0.077	
			803G	0.231	
Infants with interstitial pneumonia; Polish	Hepatopathy, skin lesions, blood dyscrasias	RFLP	857A	0.077	Zielinska, 1998 [21]
			n = 29	n = 19	
			481T	0.397	
			590A	0.328	
HIV-infected patients; UK	Rash with or without fever	RFLP	803G	0.103	Pirmohamed, 2000 [17]
			857A	0.103	
			n = 56	n = 89	
			481T	0.473	
HIV-infected patients; UK	Fever and maculopapular rash	RFLP	590A	0.348	Alfirevic, 2003 [49]
			803G	0.446	
			857A	0.018	
			n = 32	n = 8	
HIV-infected patients; UK	Fever and maculopapular rash	RFLP	481T	0.406	Alfirevic, 2003 [49]
			590A	0.344	
			803G	0.406	
			857A	0.000	

Population	Sulfonamide HS manifestations	Genotyping method	Minor allele frequency ¹		Publication
			HS	TOL	
Immuno-competent adults; Caucasian American	Primarily rash with or without fever	Direct re-sequencing	n = 99	n = 99	
			481T	0.455	481T 0.399
			590A	0.252	590A 0.308
			803G	0.495	803G 0.414
			857A	0.025	857A 0.010

¹ As reported, or as estimated from raw data or reported haplotypes.

² Restriction fragment length polymorphism.