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The Influence of Pharmacogenetic Variants in HIV/Tuberculosis Co-infected Patients in Uganda in the SOUTH Study

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Abstract

Unsatisfactory treatment outcomes have been reported in HIV/tuberculosis (TB) co-infected patients. Aim of this study was to assess the influence of single nucleotide polymorphisms (SNPs) in genes encoding for proteins involved in antitubercular drug disposition or effect.

A pharmacogenetic study was conducted in Kampala, Uganda, where all analysis were performed. The impact of SNPs on antitubercular drugs exposure, adverse events and treatment outcomes was evaluated in HIV/TB co-infected subjects receiving treatments for both conditions.

In 221 participants NAT2 (rs1799930), SLCO1B1 (rs4149032) and PXR (rs2472677) variants affected isoniazid exposure in multivariate analysis. Most patients were deemed cured (163, 73.8%), yet PXR 63396TT carriers had a higher probability of death (p=0.007) and of worsening peripheral neuropathy (p=0.018).

In this exploratory study in Ugandan HIV/TB coinfected patients genetic variants in *PXR*, *SLCO1B1* and *NAT2* were moderately associated with isoniazid exposure while *PXR 63396TT* carriers showed worse outcomes.

Introduction

With 9.6 million new cases/year and approximately 1.5 million deaths/year, tuberculosis (TB), is a one of the leading infectious disease in terms of morbidity and mortality worldwide.(1) It is the most frequently occurring opportunistic infection in people living with HIV and it is associated with significant attributable mortality. For example, HIV/TB co-infected patients have worse treatment outcomes and higher relapse rates compared to HIV negative patients with TB.(2)(3)(4). Suboptimal plasma concentrations of anti-TB drugs are commonly found in HIV-positive patients that may be due to unpredictable drug absorption and potential drug-drug interactions.(5) Low serum concentrations of antitubercular drugs are associated with slow response and treatment failure and they may contribute to the selection of drug-resistant strains.(6) A recent analysis in TB/HIV co-infected patients in Uganda showed that patients with low concentrations of rifampicin (RIF) and isoniazid (INH) were less likely to reach sputum culture conversion before the end of TB treatment or by the end of follow up (6 months after the completion of treatment).(7)

Inter-individual variability, tissue penetration and drug-drug interactions are partially explained by genetic variants in genes encoding for drug metabolizing or transporting proteins. Single nucleotide polymorphisms (SNPs) are the most common genetic variants; they seldom influence protein expression or activity while some genetic variants may affect the risk of developing certain diseases as well as the efficacy and tolerability of several compounds. (8)(9)

One of the first observed genetic variants of clinical consequence was the association between acetylator state (slow versus fast) and INH toxicity.(10) Beyond polymorphisms of N-acetyltransferase 2 (*NAT2*) gene, several other SNPs have been associated with anti-TB drugs' exposure and toxicity. For instance genetic variants in solute carrier organic anion transporter family member 1B1 (*SLCO1B1*, encoding for Organic Anion Transporter 1B1 or OATP1B1) were associated with lower RIF concentrations in African patients.(11)(12) Additionally, SNPs in carboxylesterase 2 (*CES2*) gene were associated with RIF exposure while rare variants in P-glycoprotein encoding gene (*ABCB1*) with the prevalence of drug-resistant *Mycobacterium tuberculosis* strains.(13)(14)

Co-administration of antiretrovirals further complicates the treatment of TB in HIV-positive patients. Efavirenz (EFV), a widely used non-nucleoside reverse transcriptase inhibitor, is a substrate of cytochrome P450 2B6 (CYP2B6) and RIF is a potent inducer that can affect EFV serum concentrations. However, wide interindividual variability was observed in patients receiving EFV and RIF and variants in several genes (CYP2B6, CYP3A4, CYP2A6, UGT1B27) have been associated with the degree and direction of this drug drug interaction.(15)(16) INH may also take part in this process since it has been shown that it may inhibit several CYPs at clinically relevant concentrations.(17) EFV-associated neuropsychiatric effects have been reported in patients with high serum concentrations and with certain SNPs, thus providing an impetus for exploring a role for personalized medicine.(18)

This study aimed to investigate the association between the patients' pharmacogenetic profiles and pharmacological and clinical outcomes in HIV/TB coinfected patients in Uganda.

Results

Two hundred and twenty-one samples (from 221 patients, out of 268 from the main protocol) were available and they were included in this analysis (between May 2013 and November 2015). One hundred and thirty participants (58.8%) were male; median age and weight were 34 years (IQR 29-40) and 50.2 Kg (IQR 46-57). Baseline and nadir CD4+ cell count were 161 (IQR 45-277) and 154

(IQR 47-267) cells/µL, respectively. The majority of patients fell into WHO stages III (193, 89.8%) and IV (5, 7%). Sputum-positive smears were recorded in 160 patients (72.4%).

Prevalence of genetic variants

Table 1 depicts the prevalence of the studied genetic variants. The results for *HNF4A* and *VDR820* SNP were not available in 30 and 31 patients, respectively. All SNPs were in Hardy-Weinberg equilibrium, except for *SLCO1B1* 032 and *NAT2**6 SNPs.

Pharmacokinetics

Maximal plasma concentrations (C_{max}) and areas under the curve between 0 and 4 hours (AUC_{0-4}) are shown in Table 2. A significant decrease in INH and RIF AUC_{0-4} were observed at week 8 (as compared to week 2, p=0.001 and 0.020, respectively).

We observed a significant correlation between *NAT2**6 (higher AUCs), *SLCO1B1* (lower AUCs) and *PXR* (higher AUCs) genetic variants and INH Cmax and AUC at week 8. INH exposure at week 8 according to gender and SNPs is shown in Figure 1 (AUCs) and in ~~Supplementary~~ Figure S1 (Cmax).

A multivariate linear regression analysis was performed including gender, weight and the three SNPs: female gender (p=0.013, Beta= 0.18, 95%CI 0.18-1.57), *NAT2* *6 (p=0.009, Beta= 0.19, 95%CI 0.20-1.34), *SLCO1B1* 032 (p=0.015, Beta= -0.18, 95%CI -1.01-0.11) and *PXR* 63396 (p=0.005, Beta= 0.21, 95%CI 0.22-1.25) genetic variants were independent predictors of INH AUC0-4 at week 8.

The percentage of patients showing INH C_{max} above 3000 ng/mL at week 8 was moderately predicted by genetic variants in *NAT2* *6 [7.1% (GG) vs. 17.1% (GA) vs. 28.6% (AA), p=0.067], *SLCO1B1 032* [19% (TT) vs. 10.5% (TC) vs. 0% (CC), p=0.029] and *PXR* 63396 [8.3% (CC) vs. 11.6% (CT) vs. 27.3% (TT), p=0.066].

We observed no statistically significant association of the other studied SNPs with drugs exposure.

Treatment associated toxicity

Liver toxicity was observed in 77 participants out of 211 (36.5%). ALT elevations were mostly mild (32, 15.2% at week 2 and 27, 14.1% at week 8) or moderate (5, 2.4% at week 2 and 1, 0.5% at week 8); grade 3 and 4 were observed in 4 and 3 individuals, respectively.

Any ALT elevation under treatment was more commonly observed in patients with the CC variant in *SLCO1B1* gene (53.1% vs. 32.9%, p=0.029, OR 2.306, 95%CI 1.075-4.948) (Supp Figure S2 - left). Additionally, ALT elevations at week 8 were higher in patients with the TT variant in *PXR* gene (with 4.17%/4.17% versus 0%/0.59% grade 2/3 elevations, p=0.021).

In patients with baseline ALT <40 UI/mL, ALT values had a significantly higher increase in those with the CYP2B6 TT variant [+16 UI/mL (4-29) and a 1.8 fold (0.4-3.6) increase] versus those with the GG/GT genotypes [+7 UI/mL (-1+14) and a 0.4 fold (0-1.5) change] (p=0.043 and p=0.016, Supp Figure S2 - right).

Worsening peripheral neurotoxicity was reported in 29 (13.7%) patients: the majority were grade 1 (25, 86.2%) while few were grade (3, 10.3%) and only one patient presented with grade 3 symptoms (3.4%). It was observed more frequently in patients with the TT variant in *PXR* gene (26.7% vs. 11.0%, p=0.018, OR 2.944, 95%CI 1.164-7.443) (Figure 2).

We observed no statistically significant association of the other studied SNPs with liver toxicity or peripheral neuropathy.

Outcomes

Most patients were deemed cured (163, 73.8%) with very few failures/relapses (7, 3.2%) and default cases (1, 0.5%); we observed 13 deaths (5.9%) and 18 individuals were lost to follow up (8.1%). Causes of death included worsening opportunistic conditions with potential IRIS (4 Cryptoccoal meningitis, 1 massive haemoptysis), sudden cardiac death (1), acute kidney injusry (1), sepsis (1) and gastrointestinal bleeding (1) while no information was available in 4 individuals.

In 159 patients with positive baseline sputum smears and negative samples at follow up, sputum conversion was obtained after a median of 55 (15-57) days. At log-rank analysis using Kaplan-Meier curves we observed no effect of genetic variants on time to sputum conversion.

Variants in *PXR* gene were associated with a non-significantly lower chance of achieving TB cure (66.7%) with patients presenting a TT genotype at position 63396 versus individuals with CC/CT alleles (74.9%) (p=0.342); this was driven by a significantly higher probability of death in individuals presenting the TT 63396 SNP in *PXR* (16.7%) versus those presenting other variants (4.2%) (p=0.007, OR 4.575, 95%CI 1.388-15.083) (Figure 3).

We observed no statistically significant association of the other studied SNPs with treatment associated outcomes.

Discussion

Globally, treatment of HIV/TB co-infected patients is complicated by drug-drug interactions, pill burden, adverse events and poorer outcomes. The efficacy of anti-TB treatment is of utmost importance to prevent the risk of selection and transmission of *Mycobacterium tuberculosis* resistant strains in the high-risk group of HIV-positive subjects.(19)

In this study, we observed that genetic variants in genes encoding for proteins implicated in the metabolism and transport may moderately influence the pharmacokinetics, efficacy and tolerability of drugs used for treating HIV/TB co-infection. If confirmed in studies with a larger sample size and in different settings these observations may accelerate development of individually tailored treatments even in limited-resource countries. Of note all genetic tests were performed in Uganda by trained local lab technicians thus limiting the costs and potential ethical concerns of sending genetic material abroad. We did not include a cost analysis but the raw cost of analyzing three SNPs on whole blood can be currently estimated at 28.5$ (considering a full 96 wells plate for the analysis). Although we are not aware of cost-efficacy analysis considering pharmacogenetic markers, it should be highlighted that the cost of treating TB/HIV coinfected patients was estimated to be almost double of the amount used for TB patients.(20)

The pharmacokinetics of anti-TB compounds has been largely studied over time and thresholds have been defined.(21) Several studies have recognized the role of underexposure of one or more compounds and the association of this event with unfavorable outcomes such us slow response or treatment failure. In the same study population Sekaggya-Whiltshire and colleagues found a significant association between low concentrations of RIF and INH and delayed culture conversion and concluded that this may have implications for TB transmission.(7) In this pharmacogenetic analysis we observed that INH exposure (both as $AUC_{0\text{-}4}$ and C_{max}) could be predicted by gender and by genetic variants in three genes: *NAT2*, *SLCO1B1* and *PXR*. While the first two factors (gender and *NAT2* genotype) have been repeatedly reported, the other two (*PXR* and *SLCO1B1* genetic variants) are novel findings. It should be noted that after correcting for multiple comparisons, NAT2 genotype retained a significant effect on INH AUC and Cmax. INH is not known to be a substrate of OATP1B1, consequently our observation can either be by chance or because the studied SNP in *SLCO1B1* can be in linkage disequilibrium with other relevant genetic loci or this effect can be mediated by RIF intracellular concentrations. The influence of SNPs in *SLCO1B1* have been reported in several, but not all, studies and we were not able to replicate this observation. The co-administration of EFV, known to affect RIF and INH exposure, may partially explain this discrepancy as almost all previous studies were conducted in TB mono-infected subjects: the only study conducted in 56 HIV/TB coinfected patients reported the effect of *SLCO1B1* SNP on RIF concentrations at 2.5 hours, but 95% of enrolled individuals had less common genetic variants.(22) PXR has many effects during the treatment of TB as its activation regulates drug metabolizing enzymes and transporters and this effect has been associated with RIF use.(23) While Chigutsa and coll. found no effect of variants in *PXR* gene on INH exposure, PXR-induced CYP-mediated metabolism has been described.(24)

Among patients with TB, age over 35 years, female gender, elevated pre-treatment liver function tests, malnutrition and HIV infection increase the high risk of hepatotoxicity.(25) In HIV-positive patients an additional risk factor is hepatotoxicity associated with antiretroviral drugs: in our study all patients received efavirenz that has been linked to a possible, yet uncommon, liver damage.(26) The higher ALT elevation in patients carrying *CYP2B6* 516TT suggests that higher EFV exposure may contribute to the observed mild hepatic impairment in the patients we enrolled.

Additional pharmacological/pharmacogenetic factors have been identified such as EFV exposure, the presence of less common variants in *NAT2*, and, recently, in *ABCB1* and *CYP2B6* genes, while the plasma exposure of antiTB drugs does not seem to be relevant.(27) Although we reported frequent elevation of ALT in patients treated for HIV/TB, they were mostly grade 1 and very few were grade 3 or 4: therefore our ability to assess clinically relevant hepatic toxicity was low; additionally we did not collect HBV and HCV coinfections, among the possible concomitant factors in ALT elevations after cART introduction.(28) We however observed a slightly higher proportion of patients with ALT elevations with the less common variant in *SLCO1B1* and a slightly higher severity at 8 weeks in those presenting a less common variant in *PXR*. The first finding is somehow surprising since a less expressed OATP1B1 is associated with lower intra-hepatic exposure of RIF. Since several studies demonstrate that liver injury is not linked to plasma exposure our initial hypothesis was that alternative causes (such as hepatic or intracellular concentrations or specific metabolites) may link genetic variants to liver toxicity.(29) On the contrary PXR has been shown to modulate hepatotoxicity associated with RIF and INH co-therapy in a mouse model.(30) Although the exact mechanism has not been clarified, it may be related to the induction of CYPs (as shown by the presence of anti-INH, anti-CYP2E1, anti-CYP3A4 and anti-CYP2C9 antibodies) or through the PXR-mediated perturbation of heme biosynthesis.(31) When considering patients with normal baseline ALT we observed that TT genotype in *CYP2B6* was associated with a higher increase in liver enzymes: this may due the effect on efavirenz concentrations or to the involvement of CYPs in INH metabolism.

Furthermore we observed a moderately higher risk of worsening of peripheral neuropathy in patients with less common variants in *PXR* gene. Previous evidence suggested concomitant factors (such as low CD4 cell count and malnutrition), INH exposure and NAT2 metabolism as risk factors although the effect of plasma exposure of antiTB drugs is less certain.(32)

The outcomes of TB treatment are relevant for individuals' cure and for the prevention of the infection spread to others. The effect of adequate plasma exposure of anti-TB drugs is supported by several pieces of evidence and our colleagues confirmed this in the same cohort in which we studied pharmacogenetics. In patients with less common variants in *PXR* gene we observed a 3 times lower chance of curing TB and a 4.5 times higher risk of death (despite adjusting for multiple comparisons), while no effect of other SNPs (including vitamin D receptor, previously associated with treatment outcomes). This is a novel finding and the complexity of PXR influence on cellular mechanisms may explain it. From a pharmacokinetic point of view PXR regulates the expression of genes encoding several proteins involved in drug metabolism and transport: their expression may vary among organs tissues and therefore PXR activation status may have a differential impact (in macrophages versus lung tissue).(33) P-gp, for instance, is highly expressed in lung tissues and alveolar macrophages (34) and its expression may be more relevant for pulmonary concentrations rather than plasma levels. In human tissues and in a mouse model, PXR activation has been shown to reduce the effect of RIF.(35) Several pathways leading to inflammatory processes have been shown to be controlled by PXR, thus, potentially being variable among individuals in terms of appropriate immune response and microbes' killing.(36) The high heterogeneity in causes of death did not allow for exploring the association for specific unfavorable outcomes.

Allele frequency was similar to published data from East Africa for *NAT2*6* (26% vs. 24-33) but lower for *ABCB1 3435(8.6% vs. 57-84%)* but genetic heterogeneity has been reported to be high among different ethnic groups even within the same region.(37) Yet allele frequency was intermediate for the three candidate genes [*PXR 63396* (39%), *SLCO1B1 032* (33%) and *NAT2*6* (26%)] thus supporting their potential use in this clinical setting. It should be noted that NAT2*6 and SLCO1B1 032 variants were not in Hardy-Weinberg equilibrium, thus suggesting a hypothetical evolutionary benefits that need to be further confirmed.

The limited sample size (that was driven by the original study sample determination and by available samples) should be acknowledged as a limitation for the study power to detect clinically relevant outcomes. Yet applying sample size calculation post hoc with the observed difference in INH AUC between PXR 63396 CC/CT and TT carriers (-1.53 with a SD of 2.33), and using -an alpha error of 0.05, a power of -0.89 was obtained.

In conclusion we observed that genetic variants in *PXR* affected INH metabolism (along with gender, *NAT2* and *SLCO1B1* SNPs)~~ and peripheral neuropathy~~; we report here a detrimental effect of the same polymorphisms on HIV/TB coinfected patients' survival warranting further studies in independent cohorts.

Methods

A pharmacogenetic sub-study was carried out within the SOUTH cohort study (Study on Outcomes related to TB and HIV drug Concentrations in Uganda). In this prospective observational study the correlation of anti-TB drug concentrations and clinical response in HIV-infected individuals with new pulmonary TB was investigated. The study was conducted at the Infectious Diseases Institute (IDI), Kampala, Uganda, a center of excellence in HIV care and treatment. (7)(38)

Ethics approval was received from the Joint Clinical and Research Centre Institutional Review Board, the Uganda National Council for Science and Technology and the National Drug Authority. Written informed consent was obtained from all study participants prior to enrolment and separate consent was obtained for pharmacogenetics study participation. This study was registered at Clinicaltrials.gov (NCT01782950).

The complete SOUTH study protocol is detailed elsewhere.(38)

Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

a) Evidence of a personally signed and dated informed consent document indicating that the subject (or a legal representative) has been informed of all pertinent aspects of the study;
b) Subjects who are willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other study procedures;
c) Age of ≥18 years;
d) First episode of pulmonary TB i.e. proven or highly suspected TB considered for TB treatment qualifying for 6 months anti-Tb drugs regimen (2 months RZHE and 4 months RH);
e) Confirmed HIV-1 infection .

Subjects presenting with any of the following will not be included in the study:

a) Unable to provide informed consent
b) Documented or highly suspected TB infection of any organs/systems other than the lung requiring TB treatment longer than 6 months
c) Previously treated for a mycobacterial infection (TB or atypical mycobacterial infection, active or latent)
d) Pregnancy or planned pregnancy within the next year

e) Unwillingness to perform pregnancy test

f) Decompensated liver disease and/or aminotransferases >5x ULN

g) GFR < 50ml/min

h) Co-morbidities reducing life expectancy to <1 year (e.g. cancer)

i) Patient wishes to take part in another interventional study

First-line TB treatment was administered according to WHO guidelines consisting of RIF (10 mg/kg), INH (5 mg/kg), ethambutol (ETB, 15 mg/kg) and pyrazinamide (PZA, 20 mg/kg) for 8 weeks, followed by RIF and INH for 16 weeks. In treatment-naïve patients, antiretroviral treatment (ART) consisting of tenofovir, lamivudine and EFV was started at least two weeks after initiation of TB treatment. Patients who were already on ART at the time of TB diagnosis continued their treatment while patients on nevirapine were switched to EFV to reduce drug-drug interactions. Patients on protease inhibitors received rifabutin instead of RIF, and were therefore excluded from this analysis.

Serum concentrations of RIF, INH, ETB and PZA were measured at 1, 2 and 4 hours post-dosing. EFV (600 mg) was measured at 12±2 hours post-dosing, but the results of genetic variants and EFV exposure have been reported separately.(39) Patients with undetectable 2-hour concentrations were excluded from the analysis. The pharmacokinetic analyses were performed with HPLC (High Performance Liquid Chromatography) and AUCs were estimated using non-compartmental analysis.

SNPs were analyzed through real-time PCR by allelic discrimination. Genes were selected following the available evidence of an effect of the encoded proteins on antitubercular drugs exposure (Pg-P, OATP1B1, NAT2) or treatment response (VDR), on efavirenz exposure (CYP2B6) or on the theoretical intracellular pathways that have been involved in drug metabolism and activity (PXR, HNF4-alpha).

The following genes_SNPs were studied: *ABCB1 3435 C>T (rs1045642), SLCO1B1 032 T>C (rs4149032), NAT2*6 G>A (*rs1799930*), CYP2B6 516 G>T (*rs3745274*), PXR 63396 C>T* *(*rs2472677*), HNF4α 975 C>G (*rs1884613*), VDR Cdx2 A>G (*rs11568820*).* They were selected following previous data involving either a significant effect on the enzyme expression/activity, on pharmacokinetics or treatment effect in patients with TB and taking into account the prevalence of less common variants. For instance *NAT2*6* was chosen because the reported prevalence of allele frequency in East Africa was 24-33%.(40)

We were not able to assess the haplotype frequency in Uganda for several genes because data are limited (no published study reported the prevalence of PXR SNPs) and genetic heterogeneity wide.

All pharmacokinetic and pharmacogenetic analyses were performed at the IDI research translational laboratory in Kampala: SNP results were available after the study completion and therefore retrospectively analyzed.

Severity of hepatotoxicity was graded according to the National Institutes of Health Division of AIDS toxicity tables as follows: ALT 40–119, mild/grade 1; ALT 120–199, moderate/grade 2; ALT ≥200, severe/ grade 3. Peripheral neuropathy was evaluated clinically and reported in those patients complaining of worsening symptoms under treatment and graded according to DAIDS toxicity tables.

Descriptive data are presented as median with interquartile range [IQR] for continuous variables, and as numbers and percentages for categorical variables. AUCs were calculated using Kinetica version 5.1 SP1 (Thermo Fisher Scientific Inc. 2014). Kruskal Wallis test by ranks, the non-parametric version of analysis of variance, was used to test equality of AUCs medians among polymorphisms of investigated SNPs. All the analysis were performed with STATA software, version 13.1 and SPSS version 25. For assessing the effect of SNPs on plasma exposure we applied Bonferroni's correction and set the threshold of p values at 0.007 (0.05 divided by 7).

Study Highlights

What is the current knowledge on the topic?

The treatment of tuberculosis in HIV-positive patients is challenging and it has been associated with lower success rates as compared to HIV-uninfected patients. Several genetic variants have been associated with antitubercular drugs plasma concentrations and liver toxicity but their use in clinical practice is extremely limited.

What question did this study address?

This study addresses the impact of genetic variants in selected genes involved in drugs metabolism or transport on antitubercular drugs pharmacokinetic profile, liver or neurological toxicity and outcomes. This was studied in a population of HIV-positive patients with active tuberculosis in Uganda.

What does this study add to our knowledge?

The study suggests that genetic variants (assessed in a Uganda-based laboratory) in genes encoding for Pregnane-X-receptor, Organic Anion Transporter Protein 1B1 and N-Acteyl-Transferase 2 are associated with isoniazid exposure. The association of *PXR 63396 TT* variant with an increased risk of death and worsening peripheral neuropathy is novel but needs to be confirmed in independent studies.

How might this change clinical pharmacology or translational science?

Once confirmed in other studies these data may support the use of genetic markers for antitubercular tailored treatment in hard-to-treat patients.

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Author contributions

AC and IM Wrote Manuscript. JC, CS-W, AvB, BC, JF and ML Designed Research, JC and GT Performed Research, AC, IM and GDP Analyzed Data, and JC and GDP Contributed New Reagents.

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Figure Legends

Figure 1. Isoniazid plasma Area under the Curve (0-4 hours) according to gender and genetic variants in transporters and metabolizing enzymes. Bold horizontal lines represent median values while smaller lines and whiskers depict interquartile ranges.

Supplementary Figure 1. Isoniazid plasma maximal concentration (Cmax, 2 hours after dosing) according to gender and genetic variants in transporters and metabolizing enzymes. Bold horizontal lines represent median values while smaller lines and whiskers depict interquartile ranges.

Figure 2. Prevalence of worsened peripheral neuropathy according to PXR genotype.

Supplementary Figure 2. Change in ALT levels according to genotypes in *SLCO1B1* (left, any elevation in ALT levels above 40 UI/mL) and in *CYP2B6* (right, ALT fold change in respect to baseline values).

Figure 3. Prevalence of different outcomes according to *PXR 63396* genotype.