Whole-Exome Sequencing Reveals Defective CYP3A4 Variants Predictive of Paclitaxel Dose-Limiting Neuropathy

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Abstract

Purpose: Paclitaxel, a widely used chemotherapeutic drug, can cause peripheral neuropathies leading to dose reductions and treatment suspensions and decreasing the quality of life of patients. It has been suggested that genetic variants altering paclitaxel pharmacokinetics increase neuropathy risk, but the major causes of interindividual differences in susceptibility to paclitaxel toxicity remain unexplained. We carried out a whole-exome sequencing (WES) study to identify genetic susceptibility variants associated with paclitaxel neuropathy.

Experimental Design: Blood samples from 8 patients with severe paclitaxel-induced peripheral neuropathy were selected for WES. An independent cohort of 228 cancer patients with complete paclitaxel neuropathy data was used for variant screening by DHPLC and association analysis. HEK293 cells were used for heterologous expression and characterization of two novel CYP3A4 enzymes.

Results: WES revealed 2 patients with rare CYP3A4 variants, a premature stop codon (CYP3A4*20 allele) and a novel missense variant (CYP3A4*25, p.P389S) causing reduced enzyme expression. Screening for CYP3A4 variants in the independent cohort revealed three additional CYP3A4*20 carriers, and two patients with missense variants exhibiting diminished enzyme activity (CYP3A4*8 and the novel CYP3A4*27 allele, p.I475V). Relative to CYP3A4 wild-type patients, those carrying CYP3A4 defective variants had more severe neuropathy (2- and 1.3-fold higher risk of neuropathy for loss-of-function and missense variants, respectively, $P = 0.045$) and higher probability of neuropathy-induced paclitaxel treatment modifications (7- and 3-fold higher risk for loss-of-function and missense variants, respectively, $P = 5.9 \times 10^{-5}$).

Conclusion: This is the first description of a genetic marker associated with paclitaxel treatment modifications caused by neuropathy. CYP3A4 defective variants may provide a basis for paclitaxel treatment individualization. Clin Cancer Res; 21(2); 322–8. ©2014 AACR.

Introduction

Paclitaxel is an antimitotic agent widely used for the treatment of many solid tumors. Peripheral neuropathy is the major toxicity limiting the clinical utility of this drug (1, 2). The degree of neuropathy is highly variable among patients, and while some remain asymptomatic throughout treatment, those with severe neuropathy can require paclitaxel dose reductions and treatment suspension, and therefore receive potentially suboptimal treatment. The most severe cases sustain long-term damage to the peripheral nerves, substantially reducing their quality of life (3).

Paclitaxel-induced neuropathy is dose dependent, and there are various clinical conditions that have been suggested as risk factors, such as diabetes mellitus, chronic liver disease, alcoholism, and previous neuropathies (4). Genetic variation has also been suggested as a factor influencing neuropathy risk, based on both genome-wide association studies (5, 6) and candidate gene approaches focused on paclitaxel pharmacokinetics (7). A correlation between the severity of the neuropathy and paclitaxel levels in plasma has been described (8, 9). In fact, it has been shown that common polymorphisms in the two genes encoding paclitaxel metabolizing enzymes in the liver, CYP2C8 and CYP3A4, are associated with a moderately increased risk of developing...


Translational Relevance
Paclitaxel is a cytotoxic agent widely used for the treatment of many cancers. Treatment with this drug frequently results in peripheral sensory neuropathy that can seriously affect patients’ quality of life. We and others have shown that variant alleles moderately decreasing the expression of genes involved in paclitaxel metabolism (i.e., CYP2C8*3 or CYP3A4*22) are associated with paclitaxel-induced neuropathy. The identification of predictive genetic markers for paclitaxel-induced dose-limiting neuropathy could lead to individualized risk assessment, facilitating treatment decision-making and therefore being of great clinical value. In this study, whole-exome sequencing of severe paclitaxel-induced peripheral neuropathy patients, we confirm the earlier described implication of CYP3A4 in paclitaxel-induced neuropathy and find an association of CYP3A4 defective variants with paclitaxel treatment modifications. This study emphasizes the need to screen for rare genetic variants in selected cohorts of patients and may provide a basis for paclitaxel treatment individualization.

Patients and Methods
Materials used
Dibenzylfluorescein (DBF), fluorescein, paclitaxel, 3p-hydroxypaclitaxel (3OH-P), NADPH, glucose-6-phosphate, and yeast glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich (Sigma-Aldrich Sweden AB). Lipofectamine LTX/PLUS and cell medium were purchased from Invitrogen (Life Technologies). Lipid analysis by high-performance liquid chromatography (LC-MS) was performed by Eurofins 

Paclitaxel-induced neuropathy during paclitaxel treatment (CYP2C8*3 and CYP3A4*22 alleles) (8, 10, 11). The identification of predictive genetic markers for paclitaxel-induced dose-limiting neuropathy could lead to individualized risk assessment, facilitating treatment decision-making and therefore being of great clinical value.

The newly developed whole-exome sequencing (WES) technology facilitates the identification of mutations and rare variants in exons and exon/intron boundaries that may potentially be implicated in disease and in extreme phenotypes (12–16). Thus, WES could be applied to unveil novel high-impact alleles of importance for individual variation in drug metabolism and adverse drug effects. In this study, we identified a loss-of-function allele and a novel missense variant in the CYP3A4 gene among 8 patients with severe paclitaxel-induced neuropathy. Further screening for CYP3A4 variants in an independent patient cohort revealed additional loss-of-function and missense allele carriers. Patients with CYP3A4 defective variants had higher risk of neuropathy and a large increased risk of paclitaxel dose reductions or treatment suspensions. These results highlight the fact that genetic variants that are rare in the general population might be more prevalent in patient groups developing adverse drug reactions and indeed constitute pharmacogenomic biomarkers of value for individualized therapy.

Table 1. Characteristics of the 8 patients included in WES

<table>
<thead>
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<th>Characteristic</th>
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<tr>
<td>Age (y)</td>
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<tr>
<td>Tumor stage</td>
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<td>First-line chemotherapy treatment</td>
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<td>Chemotherapy cycles</td>
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<td>Min-max</td>
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<tr>
<td>Paclitaxel dose at grade 3 sensory neuropathy (mg/m²)</td>
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<tr>
<td>Median (min–max)</td>
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<td>Median (min–max)</td>
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<tr>
<td>Paclitaxel treatment modifications due to neuropathy⁹</td>
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<td>Treatment suspension</td>
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<td>Dose reduction</td>
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Notes:
²Unless otherwise indicated.
³FEC+T: 5-fluorouracil 600 mg/m², epirubicin 90 mg/m², and cyclophosphamide 600 mg/m², every 21 days, followed by paclitaxel 100 mg/m², every 7 days.
⁴FEC+T+H: 5-fluorouracil 600 mg/m², epirubicin 90 mg/m², and cyclophosphamide 600 mg/m², every 21 days, followed by paclitaxel 100 mg/m² and herceptin (6 mg/kg loading dose; 2 mg/kg subsequent doses), every 7 days.
⁵FEC75–T80: 5-fluorouracil 600 mg/m², epirubicin 75 mg/m², and cyclophosphamide 600 mg/m², every 21 days, followed by paclitaxel 80 mg/m², every 7 days.
⁶T+H: paclitaxel 80 mg/m² plus herceptin (4 mg/kg loading dose; 2 mg/kg subsequent doses), every 7 days.
⁷Duration of grade 2 or 3 sensory neuropathy after finishing paclitaxel treatment.
⁸When the same patient paclitaxel dose was reduced and later treatment was suspended, the patient is included in the table as “treatment suspension.”

Dose reductions of paclitaxel were defined as follows: 75% of the previous dose (FEC75), 50% of the previous dose (FEC50), or a complete dose suspension (FEC0). Paclitaxel treatment modifications due to neuropathy were as follows: dose reduction (≥2-fold drop in dose), dose suspension (≥ ≥2-fold drop in dose), and complete dose suspension (FEC0).

Prevalent in patient groups developing adverse drug reactions and previous neuropathies, were ruled out. DNA from an independent cohort of 228 patients with breast and ovarian cancer treated with paclitaxel and recruited in different Spanish hospitals from Madrid, starting on January 2011, was available for genetic analysis. The primary objective of this series was to study paclitaxel-induced peripheral neuropathy, for this reason each patient had a complete neuropathy assessment. For each patient, the following information was available: demographics, tumor characteristics, maximum sensory neuropathy grade during paclitaxel treatment, neuropathy evolution once paclitaxel treatment was ceased, cumulative dose of paclitaxel, and paclitaxel dose reductions and suspensions and their causes (Supplementary Table S1).

All patients with cancer were older than 18 years of age, had documented histologic cancer neoplasia, a life expectancy of ≥12 weeks and ECOG performance status ≤2, adequate bone marrow, renal and hepatic function and no previous history of neuropathy, and had taken some form of contraception.

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Clin Cancer Res; 21(2) January 15, 2015

Published OnlineFirst November 14, 2014; DOI: 10.1158/1078-0432.CCR-14-1758

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To ensure homogeneity in neuropathy grading across the different collaborating centers, a qualified nurse (L. Sánchez) trained by a neurologist (G. Gutiérrez-Gutiérrez) interviewed by telephone all patients included in the study in a systematic manner to determine severity of symptoms and impairment in activities of daily living (e.g. extension and intensity of paresthesia, sensitivity, strength in hands and feet) and evaluated the neuropathy grade according to NCI-CTC v4. The recruitment of patients and collection of samples was approved by local internal ethical review committees and all patients gave written informed consent to participate in the study.

**WES**

WES of DNA samples from the 8 patients with extreme sensory neuropathy (Table 1) was carried out at the National Centre for Genomic Analysis (CNAG). DNA was isolated from peripheral blood using the FlexiGene DNA Kit (Qiagen) and quality control was performed according to electrophoresis and spectrophotometric measurements. The Covaris S2 System (Covaris) was used for DNA fragmentation and exome capture was performed using the SureSelect XT HumanAllExon 50Mb kit (Agilent Technologies). Library size and concentration was determined using Bioanalyzer 2100 (Agilent Technologies). Exome sequencing at a mean coverage $>50 \times$ was performed using 75-bp paired-end technology in a HiSeq2000 (Illumina). Real-time image analysis and base calling was performed using Illumina’s Real Time Analysis software version 1.6 using standard parameters. The GEM (http://algorithms.cnag.cat/wiki/The_GEM_library) and BFAST (17) programs were used to align the reads against the whole human genome (hg19 assembly). To identify single-nucleotide variants (SNV) and insertion-deletions (indels), the SAMtools program was used (http://samtools.sourceforge.net). Variants were filtered to rule out those in genome regions with low mappability, those with a strand bias $P$ value $<0.001$ in at least one sample and those with low depth read ($<15 \times$), the alternative allele present in $<20\%$ of reads, and/or the alternative allele present only in forward or reverse reads.

**CYP3A4 variant detection**

The full CYP3A4 coding region was amplified in the prospective cohort by PCR using specific primers (Supplementary Table S2). Screening for CYP3A4 variants was performed using denaturing high-performance liquid chromatography (DHPLC), in the DNA WAVE system 4500 HT (Transgenomic), equipped with a DNA-Sep column (Transgenomic). Sequencing of PCR products was performed on an ABI PRISM 3700 DNA Analyzer capillary sequencer (Applied Biosystems).

Genotyping was performed on 15 ng of genomic DNA using the KASPar SNP Genotyping System (Kbiosciences). All assays included DNA samples with known genotypes and negative controls. The Sequence Detection System ABI PRISM 7900HT (Applied Biosystems) was used to determine fluorescence and for allele assignment.

**CYP3A4 expression vectors and heterologous expression**

The coding region of CYP3A4.1 cDNA (NM_017460.5) was cloned into pcMV5 at the Xbal and Kpn1 restriction enzyme sites, to generate pcP3A4.1 plasmid. To introduce c.1165C>T and c.1423C>G variants, we used the QuickChange II Site-Directed Mutagenesis Kit (Agilent Technologies) following the manufacturer's instructions. The correct sequence of CYP3A4 variant plasmids (pcP3A4-P389S and pcP3A4-L475V) was confirmed by Sanger sequencing.

HEK293 cells were cultured in DMEM with 10% fetal bovine serum and 100 U/mL penicillin–streptomycin. Cells were transfected with pcP3A4.1, pcP3A4-P389S, pcP3A4-L475V, or empty pcMV5, together with human cytochrome b5a (pCL-cyb5) using Lipofectamine LTX/PLIIS, following the manufacturer's guidelines.

**Western blot analysis**

Cells were solubilized in RIPA buffer containing Complete Protease Inhibitor Cocktail (Roche Diagnostics). Cell lysates or microsomes, prepared as previously described (18) and containing an equal amount of total protein, were separated using 15% SDS-polyacrylamide gel (18). Membranes were probed with primary antibodies against CYP3A4 (α-hCYP3A4, 1:1,000; ref. 19), human cytochrome b5a (α-cyb5a, 1:1,000; Santa Cruz Biotechnology) and, as loading control, the housekeeping escort chaperone ERP29 (α-ERP29, 1:1,000; ref. 20). The amount of expressed CYP3A4 apoproteins was calculated by densitometric analysis (Image Gauge, v.4.0; Fujifilm) of Western blot bands using a standard calibration curve based on CYP3A4 supersomes (BD Biosciences). To determine protein stability, 48 hours post-transfection HEK293 cells were exposed to 50 µmol/L cycloheximide for 8 hours. The CYP3A4 degradation rate was estimated by immunoblottting and further densitometric analysis of protein bands.

**Determination of CYP3A4 enzyme activity**

All incubations were conducted as previously described (21, 22) with minor modifications. Briefly, microsomal fractions corresponding to 160 µg of protein were mixed with different concentrations of dibenzylfluorescein in 50 mmol/L potassium phosphate buffer (pH 7.4) and the reaction was initiated by adding a prewarmed NADPH-regenerating system. Fluorescein formation was proportional to incubation time and concentration of microsomes. After 60 minutes, formation of metabolites was measured in a SPECTRAMax Gemini microplate spectrophotometer (Molecular Devices) and results were analyzed using SoftMax Pro 5 software.

**Statistical analysis**

Michaelis–Menten constants $K_m$, $V_{max}$, and intrinsic clearance ($Cl_{int} = V_{max}/K_m$) were calculated by nonlinear regression analysis using GraphPad and statistical significance was assessed using a paired $t$ test. Association between neuropathy grade (ranked 0 to 3) and specific genetic groups was assessed using the Goodman and Kruskal Gamma test. Association between treatment modifications (binary variable) and specific genetic groups was assessed using the Fisher exact test. In these analyses, potential confounders were accounted for by stratification using the Mantel–Haenszel test. The Pearson correlation test was used to compare all genetic groups simultaneously (the genetic variable ranked in three groups: wild-type, 0; missense variants, 1; loss-of-function variants, 2) with neuropathy grade and treatment modifications. The analysis SPSS software package v.19 was used for all statistical analyses. $P$ values less than 0.05 were considered statistically significant.
Results

Detection of CYP3A4*20 and CYP3A4*11665C>T (p.P389S) variants by WES in 2 patients with extreme paclitaxel neuropathy

We first screened for loss-of-function and missense variants in critical genes involved in paclitaxel pharmacokinetics (CYP3A4, CYP2C8, ABCB1, and SLCO1B3) in 8 patients with extreme sensory neuropathy, and identified two high-impact variants in CYP3A4 (Supplementary Table S3). One was the CYP3A4*20 allele, a rare deleterious indel causing a frameshift and premature stop codon (c.1461_1462insA, p.P488Tfs*494). The other was a missense variant (c.1165C>T, p.P389S) located in the highly conserved CYP β-helix 4; it had not been reported previously, and was given the name CYP3A4*25 by the CYP allele nomenclature committee (www.cypalleles.ki.se). The patients with these variants were women treated with adjuvant FEC+T for breast cancer that upon paclitaxel treatment developed grade 3 neuropathy with loss of sensitivity in hands and feet, dysesthesia and clumsiness, and walking problems. The CYP3A4*20 carrier had two paclitaxel dose reductions due to the neuropathy and more than 40 months after paclitaxel treatment the symptoms persisted with improvement to grade 2. The CYP3A4*25 carrier had suspension of paclitaxel treatment after cycle 6 due to the neuropathy and 19 months after paclitaxel treatment symptoms had decreased to grade 2 neuropathy; 25 months after paclitaxel treatment the patient did not report neuropathy symptoms. Sanger sequencing confirmed the presence of both variants in heterozygosity (Fig. 1).

In the CYP2C8 gene, encoding the other CYP enzyme metabolizing paclitaxel, and in SLCO1B3 and ABCB1, encoding the uptake and efflux paclitaxel transporters, respectively, we only detected previously described missense variants, none of which were predicted to affect protein function. In addition, with the exception of CYP3A4, the frequency of most variants in the patients with extreme neuropathy was similar to that reported in the general population (Supplementary Table S3 shows coding polymorphisms and the regulatory intronic CYP3A4*22). Thus, we selected the CYP3A4 gene for further study.

Screening for CYP3A4 variants in a cohort of paclitaxel-treated patients

To determine whether additional CYP3A4 coding variants are carried by patients with paclitaxel-induced neuropathy, we examined by DHPLC an independent cohort of 228 patients with cancer treated with the drug (Supplementary Table S1). We detected three additional patients carrying the CYP3A4*20 allele, another patient with CYP3A4*8 allele (c.389G>A, p.R130Q), and another one with a novel CYP3A4 missense variant (c.1423C>G, p.L475V, named CYP3A4*27). The characteristics of the patients carrying CYP3A4 coding variants are shown in Table 2.

Stability and enzymatic activity of CYP3A4.25 and CYP3A4.27

The CYP3A4*20 allele has been shown to encode a non-functional enzyme (23) and the CYP3A4*8 allele was shown to cause decreased CYP3A4 activity (24), but no functional data exist on the novel missense variants CYP3A4*25 and CYP3A4*27. HEK293 cells transiently expressing CYP3A4-P389S or CYP3A4-L475V (CYP3A4.25 and CYP3A4.27, respectively) showed substantially lower amounts of protein compared with CYP3A4-wild-type (CYP3A4.1; Fig. 2). The level of the CYP3A4.27 protein in the expression system was estimated to be 10% of the corresponding expression of CYP3A4.1. The level of CYP3A4.25 was also relatively low, about 40% of the CYP3A4.1 levels and treatment of the cells with the protein synthesis inhibitor cycloheximide confirmed that the P389S substitution in CYP3A4.25 caused an increased rate of degradation (Supplementary Fig. S1). By analyzing the mRNA levels, it was found that the levels were slightly lower (about 80%–70% of the control) for the mutant variants as compared with CYP3A4.1 in the expression system (data not shown). Analyses of catalytic activities of the variant enzymes using dibenzylfluorescein as a CYP3A4 substrate revealed a similar K_{m} value for CYP3A4.27 (K_{m} 7.4 ± 1.8 μmol/L) as compared with CYP3A4.1, whereas the K_{m} value for CYP3A4.25 was somewhat higher (K_{m} 31.7 ± 2.8 μmol/L). The true V_{max} was difficult to determine because of low expression of the variant proteins (data not shown). We conclude that both CYP3A4.25 and CYP3A4.27 have decreased stability in the expression system used.

CYP3A4 variants are associated with an increased risk of neuropathy and paclitaxel treatment modifications

Thus, in a total of 236 patients, composed of 8 WES-studied patients and 228 patients used for CYP3A4 screening, 4 carried a loss-of-function variant (CYP3A4*20) and three carried rare missense variants giving rise to decreased enzymatic activity (CYP3A4*25, CYP3A4*27 and CYP3A4*8; Table 2). The patients with CYP3A4 loss-of-function variants and patients with missense variants showed a 2- and 1.3-fold increased risk of grade 3 neuropathy, respectively, when compared with wild-type CYP3A4 patients (Fig. 3A). The neuropathy grade was significantly different between patients with loss-of-function variants and wild-type homozygotes (P = 0.042), and including missense variants in the analysis only minimally changed the P value (P = 0.045). Furthermore, 14% of patients with paclitaxel dose reductions or treatment suspensions due to neuropathy carried CYP3A4 variants, and a 7- and
3-fold increased risk of treatment changes was observed in patients with loss-of-function and missense variants, respectively, when compared with wild-type CYP3A4 patients (Fig. 3B). This increased risk of treatment modifications for patients with genetically decreased CYP3A4 activity was statistically significant ($P = 5.8 \times 10^{-3}$ for loss-of-function variants, $P = 5.9 \times 10^{-3}$ when missense variants were included in the analysis). When all paclitaxel treatment modifications (i.e., including those due to reasons other than neuropathy) were considered, CYP3A4 variants were still associated with an increased risk of dose changes ($P = 7.7 \times 10^{-3}$).

Tumor type was not associated with the neuropathy, but conditions considered to be neuropathy risk factors (diabetes, high alcohol intake, restless-legs-syndrome) were significantly associated with neuropathy grade (Supplementary Fig. S2A). Cumulative paclitaxel dose was associated with treatment modifications, as expected, as these result in lower cumulative doses (Supplementary Fig. S2D). Concerning CYP3A4*22 allele, we found a trend toward higher treatment modifications in carriers of this variant ($P = 0.066$); however, no statistically significant differences were obtained for neuropathy grade and treatment modifications due to neuropathy (Supplementary Fig. S3). Accounting for neuropathy risk factors, cumulative paclitaxel dose or CYP3A4*22 allele did not substantially change the association observed for CYP3A4 defective variants.

### Discussion

Paclitaxel peripheral neuropathy affects a large number of patients and can lead to treatment modifications (25). Most patients recover from the neuropathy, but long-term nerve damage can also occur, compromising the quality of life of these patients. The extent of paclitaxel exposure is associated with the severity of the neuropathy (8, 9), and paclitaxel elimination is mediated by CYP2C8, CYP3A4, OATP1B3, and P-glycoprotein (7, 26, 27). Thus, alterations in the activity of these proteins could mediate neuropathy. Chemotherapy-induced neurotoxicity studies are challenging due to subjectivity of the common toxicity scales and the difficult application of more accurate neuropathy scales across multiple centers (36). In this study, neuropathy was assessed in a systematic manner in all collaborating centers, and for the identification of extreme-phenotype patients, not only the severity of symptoms during treatment, but also modifications of treatment regimen and long-lasting disabilities were taken into account.

WES revealed two rare high-impact variants in CYP3A4 (CYP3A4*20 and CYP3A4*25). CYP3A4*20 is an indel leading to a premature stop codon previously described in one individual with impaired elimination of CYP3A4 substrates (23) and the novel CYP3A4*25 (P389S) protein had decreased stability and reduced amounts of apoprotein in the HEK293 expression system (Fig. 2A and Supplementary Fig. S1). In an independent cohort we found three more carriers of CYP3A4*20, and two carriers of missense variants, CYP3A4*8, described to have diminished activity (24), and CYP3A4*27, here found to be less expressed in comparison with CYP3A4.1 (Fig. 2B). In total, 3% of the Spanish patients carried CYP3A4 defective variants, suggesting that these could explain part of CYP3A4 variability. Although CYP3A4*20 allele is present in Spain it has a low frequency in most European, Asian, and African populations (37) however, alternative CYP3A4 defective variants might be relevant in other populations. In this
CYP3A4 Variants and Paclitaxel Dose-Limiting Neuropathy

Figure 3.
CYP3A4 defective variants confer an increased risk of paclitaxel-induced neuropathy and treatment modifications. A, neuropathy grade was compared among patients with different CYP3A4 activity. All 4 patients with loss-of-function variants (100%), 2 of 3 patients (67%) with missense variants, and 116 of 229 patients (51%) with wild-type CYP3A4 had grade 3 sensory neuropathy. B, treatment modifications due to neuropathy were compared among patients with different CYP3A4 activity. Three of 4 patients (75%) with loss-of-function variants, 1 of 3 patients (33%) with missense variants, and 25 of 229 patients (11%) with wild-type CYP3A4 had treatment modifications. As described in Patients and Methods, the Goodman and Kruskal gamma test and Fisher exact test were used to assess association with neuropathy grade (γ = 1) and treatment modifications. To perform an analysis including simultaneously all variants categorized according to CYP3A4 activity (loss-of-function, missense, and wild-type), Pearson correlation test was used. Treatment modification due to peripheral neuropathy.

respect, 3% of European Americans and 2% of African Americans seem to carry potentially defective CYP3A4 allele variants (loss-of-function or missense variants likely damaging according to Polyphen; see Exome Variant Server database).

Patients carrying loss-of-function CYP3A4 variants had a significantly higher risk of neuropathy and paclitaxel treatment modifications, when compared with wild-type CYP3A4 patients (P = 0.042 and P = 5.8 × 10⁻³, respectively; Fig. 3), and carriers of missense variants showed an intermediate phenotype, concordant with a decreased but not abolished CYP3A4 activity. For CYP3A4*22 allele, we only detected a trend toward increased treatment modifications, which might reflect a lower effect of this variant on paclitaxel metabolism and/or a low statistical power due to the small number of CYP3A4*22 carriers (Supplementary Fig. S3). Four of the 29 patients with paclitaxel treatment modifications due to neuropathy carried CYP3A4 defective variants (missense or loss-of-function), indicating that genetic testing of CYP3A4 before treatment, would have a very high specificity (99%) but poor sensitivity (14%). If pathologic risk factors were also taken into consideration, the sensitivity would increase, and an estimated 27% of patients carrying CYP3A4 defective variants or with preexisting conditions associated with neuropathy, would require treatment modification due to severe neuropathy upon paclitaxel chemotherapy. Paclitaxel-induced neuropathy is a multifactorial and polygenetic trait, and additional genetic variants, some yet to be identified, will further improve the predictive power of genetic testing. In this regard, Supplementary Table S4 shows genetic variants that have been described as moderate risk factors for paclitaxel-induced neuropathy (CYP3A4*22, CYP2C8*3, EFHA5-rs7349683, and XRRA4-rs37264), the latter two identified in a meta-analysis of genome-wide association studies; refs. 5, 6). Thus, the identification of genetic variants and physiopathologic risk factors predictive of paclitaxel-induced neuropathy may provide a basis on which to individualize this treatment. It is important to highlight that previous reports have only identified markers associated to neuropathy grade, but not with neuropathies resulting in treatment modifications. In fact, this is the first study describing a marker associated with paclitaxel dose-limiting neuropathy.

In summary, in this study we found an overrepresentation of defective CYP3A4 variants in patients with paclitaxel treatment modifications and in those with high-grade paclitaxel-induced neuropathy. This supports and confirms the earlier described implication of CYP3A4 in paclitaxel-induced neuropathy. These results emphasize the need to screen for rare genetic variants in selected cohorts of patients.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: M. Apellániz-Ruiz, M.-Y. Lee, G. Gutiérrez-Gutiérrez, I. Calvo, J. García-Doná, M. Ingelman-Sundberg, C. Rodríguez-Antona
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Acknowledgments
The authors thank CNAG personnel and especially Sergi Beltran and Raul Tonda for their support in whole-exome sequencing. The authors also thank Lucia Inglada-Perez for her contribution to the statistical analyses.
Grant Support
This work was supported by projects from the Spanish Ministry of Economy and Competitiveness (grant number SAF2012-35793) and by grants from the Swedish Cancer Foundation, The Swedish Research Council, and Karolinska Institutet in Sweden. María Apellániz-Ruiz is a predoctoral fellow of “la Caixa”/CNIO international PhD programme.

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