# Genotype and Allele Frequencies of Drug-Metabolizing Enzymes and Drug Transporter Genes Affecting Immunosuppressants in the Spanish White Population

*Virginia Bosó, PharmD,*† María J. Herrero, PhD,*† Enrique Buso, PhD,‡ Juan Galán, MD,§ Luis Almenar, MD, PhD,¶ Ignacio Sánchez-Lázaro, MD,¶ Jaime Sánchez-Plumed, MD, PhD,‖ Sergio Bea, MD,‖ Martín Prieto, MD, PhD,** María García, MD,** Amparo Pastor, MD,†† Amparo Sole, MD, PhD,†† José Luis Poveda, PharmD, PhD,* and Salvador F. Aliño, MD, PhD*‡‡§§*

**Abstract:** Interpatient variability in drug response can be widely explained by genetically determined differences in metabolizing enzymes, drug transporters, and drug targets, leading to different pharmacokinetic and/or pharmacodynamic behaviors of drugs. Genetic variations affect or do not affect drug responses depending on their influence on protein activity and the relevance of such proteins in the pathway of the drug. Also, the frequency of such genetic variations differs among populations, so the clinical relevance of a specific variation is not the same in all of them. In this study, a panel of 33 single nucleotide polymorphisms in 14 different genes (ABCB1, ABCC2, ABCG2, CYP2B6, CYP2C19, CYP2C9, CYP3A4, CYP3A5, MTHFR, NOD2/CARD15, SLCO1A2, SLCO1B1, TPMT, and UGT1A9), encoding for the most relevant metabolizing enzymes and drug transporters relating to immunosuppressant agents, was analyzed to determine the genotype profile and allele frequencies in comparison with HapMap data. A total of 570 Spanish white recipients and donors of solid organ transplants were included. In 24 single nucleotide polymorphisms, statistically significant differences in allele frequency were observed. The largest differences (>100%) occurred in ABCB1 *rs2229109*, ABCG2 *rs2231137*, CYP3A5 *rs776746*, NOD2/CARD15 *rs2066844*, TPMT *rs1800462*, and UGT1A9 *rs72551330*. In conclusion, differences were recorded between the Spanish and other white populations in terms of allele frequency and genotypic distribution. Such differences may have implications in relation to dose requirements and drug-induced toxicity. These data are important for further research to help explain interindividual pharmacokinetic and pharmacodynamic variability in response to drug therapy.

**Key Words:** genetic polymorphism, allele frequency, immunosuppressant, CYP3A5

(*Ther Drug Monit* 2014;36:159–168)

## INTRODUCTION

Response to drug therapy varies greatly among individuals, and predicting how effective or safe a drug will be for a particular patient is not easy. Interpatient variability in drug response can be widely explained by genetically determined differences in metabolizing enzymes, drug transporters, and drug targets, leading to different pharmacokinetic and/or pharmacodynamic behaviors of drugs.

Immunosuppressants are central to pharmacological treatment after tissue or organ transplantation and are also indicated in a number of autoimmune diseases and in certain oncological or hematologic malignancies. These drugs have a narrow therapeutic index and considerable interindividual pharmacokinetic differences, which can be interpreted as a result of genetic variability.[1–3] The main metabolic enzymes and drug transporters involved in immunosuppressant drug pathways are represented in Figure 1.[4] Variations in the genes encoding for them include single nucleotide polymorphisms (SNPs) in ABCB1, which affects tacrolimus and cyclosporine response; and ABCC2, ABCG2, SLCO1A1, and SLCO1B1, which alter methotrexate and mycophenolic acid (MPA) pharmacokinetics.[5–10] CYP3A5 variants have been demonstrated to alter tacrolimus dose requirements,[11] methylenetetrahydrofolate reductase (MTHFR) gene variations have been associated with methotrexate-related toxicity,[12] and inactive TPMT alleles (*2, *3A, *3B, *3C, or *4) are associated with severe immunosuppression in thiopurine-treated patients.[13]

Genetic variations affect or do not affect drug responses depending on their influence on protein activity and the relevance of such proteins in the pharmacological pathway of the drug. Much research has been carried out to identify the

Received for publication February 2, 2013; accepted August 15, 2013.

From the *Unidad de Farmacogenética, Servicio Farmacia, Hospital Universitari i Politècnic La Fe; †Instituto Investigación Sanitaria La Fe; ‡Unidad Central de Investigación, Universidad de Valencia; §Coordinación de Trasplantes, Hospital Universitari i Politècnic La Fe; ¶Servicio de Cardiología, Hospital Universitari i Politècnic La Fe; ‖Servicio de Nefrología, Hospital Universitari i Politècnic La Fe; **CIBER on Liver and Digestive Diseases (CIBERehd), funded by the Instituto de Salud Carlos III, Madrid; de Medicina Digestiva, Hospital Universitari i Politècnic La Fe; ††Unidad de Trasplante Pulmonar, Hospital Universitari i Politècnic La Fe; ‡‡Unidad de Farmacología Clínica, Hospital Universitari i Politècnic La Fe; and §§Departamento de Farmacología, Facultad de Medicina, Universidad de Valencia, Valencia, Spain.

Supported in part by the Consellería de Sanidad, Generalitat Valenciana (grants GE-039/11, GE-007/10).

Correspondence: María J. Herrero, PhD, Unidad de Farmacogenética, Servicio de Farmacia, Hospital Universitari i Politècnic La Fe, Bulevar Sur, s/n. 46026 Valencia, Spain (e-mail: maria.jose.herrero@uv.es).

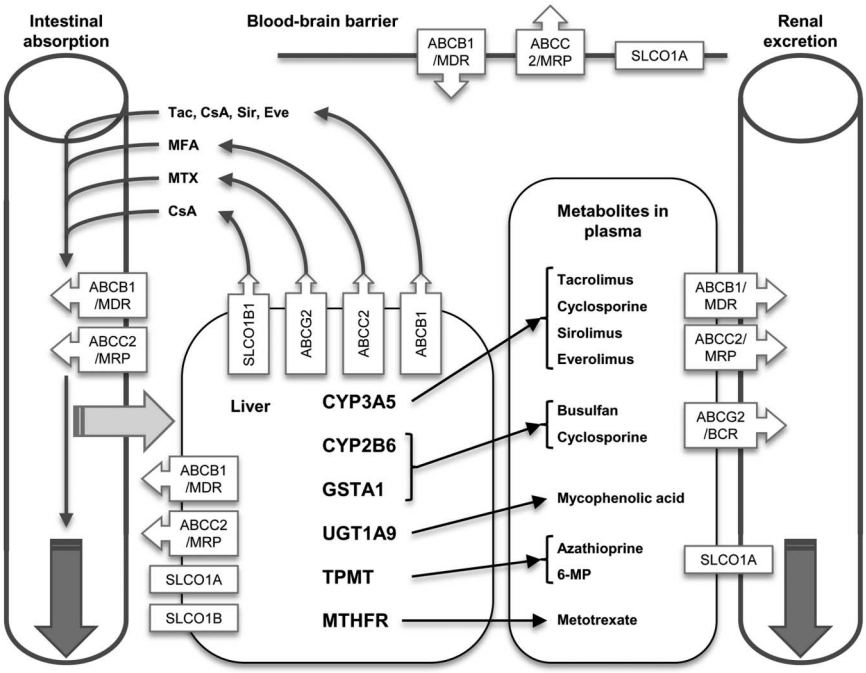


**FIGURE 1.** Major metabolic enzymes and drug transporters involved in immunosuppressant drug pathways. The figure shows the localization of the enzymes and transporters and specific drugs that are affected (modified from 4). Adaptations are themselves works protected by copyright. So to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

most relevant genetic variations involved in the response to immunosuppressants and to establish genotype–phenotype associations. Also, the frequency of such genetic variations differs among populations, so the clinical relevance of a specific variation is not the same in all of them. In fact, previous reports indicate that Spanish whites show genotype and allele frequencies for several polymorphisms in different genes, such as cytochrome P450 or ABCB1, that are different from those of other European populations.[14] Furthermore, the cost-effectiveness of a pharmacogenetic test in a given population is determined in part by the prevalence of the variation in that population.

In this study, a panel of 33 SNPs was analyzed in genes encoding for the most relevant metabolizing enzymes and drug transporters relating to calcineurin inhibitors (tacrolimus and cyclosporine), m-tor inhibitors, antiproliferative drugs (MPA and azathioprine), and other immunosuppressants such as methotrexate or cyclophosphamide, with a view to determining the genotype profile and allele frequencies in relation to immunosuppressant therapy in Spanish white solid organ transplant recipients and donors.

## MATERIALS AND METHODS

### Study Design and Patients

Between March 2008 and December 2011, all Spanish-born white solid organ transplant recipients (n = 323) and their respective donors (n = 247) who underwent surgery in our hospital and gave written informed consent were included in the study.

Genetic data were retrieved from the study population without interfering with patient management and were handled according to standard regulations for data registration and use and the preservation of patient anonymity and privacy. The study was approved by the local Clinical Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki and its amendments.

The initial immunosuppression regimen comprised a calcineurin inhibitor (tacrolimus or cyclosporine), corticosteroids, and MPA (mycophenolate mofetil or mycophenolate sodium). Azathioprine was used as second-line immunosuppression. Oral treatment was started within 24 hours of surgery unless there were complications. In maintenance immunosuppression, the calcineurin inhibitor could be switched to an m-tor inhibitor (sirolimus or everolimus).

### DNA Extraction

Genomic DNA from each sample was isolated from 200 μL of whole blood using a commercially available kit based on centrifugation in microcolumns (UltraCleanBlood-Spin DNA Isolation Kit; MoBio Laboratories Inc, Carlsbad, CA). DNA concentration and purity were measured with a NanoDrop spectrophotometer (NanoDrop Technologies Inc, Wilmington, DE). DNA was stored at −20°C until use.

### Genotyping

A total of 570 samples were genotyped. Thirty-three SNPs in 14 different genes were analyzed: ABCB1 (*rs1045642*, *rs2032582*, *rs1128503*, *rs2229109*, *rs2235013*, *rs2235033*, *rs3213619*, and *rs9282564*), ABCC2/MRP2 (*rs2273697*, *rs3740066*, and *rs717620*), ABCG2/BCRP (*rs2231137* and *rs2231142*), CYP2B6 (*rs2279343*), CYP2C19 (*rs4244285*), CYP2C9 (*rs1057910*), CYP3A4 (*rs2740574*), CYP3A5 (*rs776746*, *rs10264272*, and *rs41303343*), MTHFR (*rs1801131* and *rs1801133*), NOD2/CARD15 (*rs2066844* and *rs2066845*), SLCO1A2 (*rs11568563* and *rs11568564*), SLCO1B1 (*rs2306283* and *rs4149056*), TPMT (*rs1142345*, *rs1800460*, and *rs1800462*), and UGT1A9 (*rs6714486* and

*rs72551330*). Genotyping for these polymorphisms was carried out using the Sequenom MassArray platform. In our assays, we chose a platform conformation of a maximum of 40 markers per sample, on 40 different samples, analyzing them in triplicate, in each genotyping assay. All these markers were genotyped according to the manufacturer's instructions (Sequenom, San Diego, CA). Briefly, the SNP assay was designed using Assay Design software (www.mysequenom.com/tools). Polymerase chain reactions were carried in a 5-μL volume in a standard 384-well plate format according to the specifications provided by Sequenom. The amplified product was cleaned up using shrimp alkaline phosphatase to neutralize any unincorporated dNTPs. Allele discrimination assay reactions were conducted by adding extension primers, DNA polymerase, and a cocktail mixture of deoxyribonucleotide triphosphates and dideoxynucleotide trisphosphates to each well. MassExtend clean resin was added to the mixture to remove extraneous salts that could interfere with matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) analysis. The reaction mixture was then spotted onto a SpectroCHIP II microarray and subjected to MALDI-TOF mass spectrometry. All samples were run in triplicate, and negative controls were included as a contamination check.

## Statistical Analyses

Statistical analyses and calculations were performed using the SPSS statistical package (IBM SPSS Statistics version 19, Armonk, NY) and Prism 4 (GraphPad Software, Inc, San Diego, CA). The results were entered as frequencies, and percentages and 95% confidence intervals were calculated. The genotyping rate (successful genotyping), Hardy–Weinberg equilibrium, and minor allele frequency were calculated for each SNP. Successful genotyping assays were defined as those for which 90% of all possible genotyping calls were obtained for that SNP. The observed allele frequency distribution in our population was compared with that described in the Database of single nucleotide polymorphisms (dbSNP) (www.ncbi.nlm.nih.gov/projects/SNP/). Allele frequency data were assessed using a $\chi^2$ test. The population selected for comparisons from dbSNP was HapMap CEU (European). Other white populations were selected only if HapMap data were not available. For all the analyses, only $P$ values $<0.05$ were considered significant.

# RESULTS

Recipient distribution (n = 323) according to transplanted organ was 33.4% kidney (n = 108), 5.6% kidney–pancreas (n = 18), 18.9% heart (n = 61), 12.7% lung (n = 41), 0.3% heart–lung (n = 1), 28.2% liver (n = 91), and 0.9% kidney–liver (n = 3).

The genotyping rate was more than 95% in all cases (data not shown). All genotype frequencies were in Hardy–Weinberg equilibrium, except in the case of ABCB1 *rs2229109* and MTHFR *rs1801131*. In both these cases, the mass spectra of each of the samples were checked one by one, and no errors were found.

Genotypes and allele frequencies found in 570 Spanish white individuals for genes encoding for drug transporters and for genes codifying for metabolizing enzymes are shown in Tables 1 and 2, respectively. In these tables, the cases in which the genotyping rate did not reach 100% can be identified by the included number of samples (n) not reaching 570.

Allele distribution differences between recipients and donors were assessed ($\chi^2$ test, df = 1). Allele frequencies were very similar (data not shown), and statistically significant differences between recipients and donors were found only for CYP2B6*4 [variant (A) allele proportion of 26.0% in donors versus 34.1% in recipients, $P = 0.004$] and CYP2C19*2 [variant (A) allele proportion of 12.1% in donors versus 17.0% in recipients, $P = 0.022$]. These were the largest differences found.

In contrast, when comparing allele frequency distribution to dbSNP, statistically significant differences were found for 24 SNPs. Genotype and allele frequencies reported in dbSNP for each SNP and $P$ values are shown in Tables 1 and 2. In 2 of the SNPs, a small variant allele frequency not reported in dbSNP was described (CYP3A5*6 and CYP3A5*7). Percentage variation (%) in variant allele frequency was <10% for 7 SNPs and between 10% and 61% for 18 SNPs. Differences greater than 100% were found in 6 cases (ABCB1 1199 G > A, ABCG2 34 G > A, CYP3A5*3, NOD2/CARD15 R702W, TPMT*2, and UGT1A9 98T > C), that is in 6 of the 33 SNPs studied, the variant allele frequency in our population was over twice that described in dbSNP.

Seven different combinations of CYP3A4 and CYP3A5 genotypes were identified in the study population. Table 3 shows all the possible combinations. All the subjects carrying the CYP3A4*1B variant also carried the CYP3A5*1 allele.

# DISCUSSION

In this study, we analyzed a panel of SNPs in genes encoding for the most relevant metabolizing enzymes and drug transporters relating to calcineurin inhibitors, m-tor inhibitors, antiproliferative drugs (MPA and azathioprine), and other immunosuppressants such as methotrexate or cyclophosphamide. In human genetics, in general, but particularly in pharmacogenetic research, it is very important to obtain the most accurate allelic and genotypic data of the studied variants. The true relevance of a given polymorphism could be very different depending on the population one is working with due to the different variant frequencies found. Here, we report genotype and allele frequencies of 33 SNPs in a population of 570 Spanish white solid organ transplant recipients and donors, highlighting the differences with the European HapMap population (CEU) (dbSNP).[15,16]

Although the allele distribution between recipients and donors was very similar for the studied SNPs (only 2 of 33 SNPs presented statistically significant differences), 24 SNPs presented differences in allele distribution when compared with dbSNP.

Some SNPs in ABCB1 lead to altered P-glycoprotein expression, with important phenotypic variation of P-glycoprotein activity.[17] Compared with the HapMap CEU population,[15,16] a lesser proportion of variant allele frequency was found for 3435C > T and 2677 G > T/A and a lesser proportion of homozygote variant genotype. Variant TT genotype in 3435C > T and 2677 G > T/A has been associated with increased tacrolimus blood levels,[18,19] so dose requirements are

**TABLE 1.** Genotype and Allele Frequencies in Drug Transporters in Spanish White Organ Recipients and Donors

| | | Spanish White Population | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Genotype Frequency | | | | Allele Frequency | | |
| Gene SNP | n | | n | % | 95% CI | | % | 95% CI |
| ABCB1 *rs1045642* (3435C > T) | 569 | C/C | 154 | 27.1 | 23.6–30.9 | C | 53.0 | 50.1–55.9 |
| | | C/T | 295 | 51.8 | 47.7–55.9 | T | 47.0 | 44.1–50.0 |
| | | T/T | 120 | 21.1 | 17.9–24.6 | | | |
| ABCB1 *rs1128503* (1236 T > C) | 570 | C/C | 185 | 32.5 | 28.7–36.4 | C | 57.4 | 54.5–60.2 |
| | | C/T | 284 | 49.8 | 45.7–53.9 | T | 42.6 | 39.8–45.5 |
| | | T/T | 101 | 17.7 | 14.8–21.1 | | | |
| ABCB1 *rs2032582* (2677 G > T/A) | 569 | G/G | 200 | 35.1 | 31.3–39.2 | G | 61.0 | 58.2–63.8 |
| | | G/T | 289 | 50.8 | 46.7–54.9 | T | 37.9 | 35.1–40.7 |
| | | T/T | 69 | 12.1 | 9.7–15.1 | A | 1.1 | 0.7–1.9 |
| | | G/A | 5 | 0.9 | 0.4–2.0 | | | |
| | | T/A | 4 | 0.7 | 0.3–1.8 | | | |
| | | A/A | 2 | 0.4 | 0.1–1.3 | | | |
| ABCB1 *rs2229109* (1199 G > A) | 570 | G/G | 513 | 90.0 | 87.3–92.2 | G | 94.2 | 92.7–95.4 |
| | | G/A | 48 | 8.4 | 6.411.0 | A | 5.8 | 4.6–7.3 |
| | | A/A | 9 | 1.6 | 0.8–3.0 | | | |
| ABCB1 *rs2235013* (1725 + 38 G > A) | 568 | G/G | 132 | 23.2 | 20.0–26.9 | G | 48.6 | 45.7–51.5 |
| | | A/G | 288 | 50.7 | 46.6–54.8 | A | 51.4 | 48.5–54.3 |
| | | A/A | 148 | 26.1 | 22.6–29.8 | | | |
| ABCB1 *rs2235033* (1554 + 24T > C) | 569 | T/T | 132 | 23.2 | 19.9–26.8 | T | 48.4 | 45.5–51.3 |
| | | C/T | 287 | 50.4 | 46.3–54.5 | C | 51.6 | 48.7–54.5 |
| | | C/C | 150 | 26.4 | 22.9–30.1 | | | |
| ABCB1 *rs3213619* (-129 T > C) | 569 | T/T | 537 | 94.4 | 92.2–96.0 | T | 97.2 | 96.1–98.0 |
| | | C/T | 32 | 5.6 | 4.0–7.8 | C | 2.8 | 2.0–3.9 |
| ABCB1 *rs9282564* (61 A > G) | 570 | A/A | 503 | 88.2 | 85.3–90.6 | A | 93.9 | 92.3–95.1 |
| | | A/G | 64 | 11.2 | 8.9–14.1 | G | 6.1 | 4.9–7.7 |
| | | G/G | 3 | 0.5 | 0.2–1.5 | | | |
| ABCC2 *rs2273697* (1249 G > A) | 569 | G/G | 367 | 64.5 | 60.5–68.3 | G | 80.7 | 78.3–82.6 |
| | | G/A | 184 | 32.3 | 28.6–36.3 | A | 19.3 | 17.1–21.7 |
| | | A/A | 18 | 3.2 | 2.0–4.9 | | | |
| ABCC2 *rs3740066* (3972 C > T) | 569 | C/C | 216 | 38.0 | 34.1–42.0 | C | 61.4 | 58.6–64.2 |
| | | C/T | 267 | 46.9 | 42.9–51.0 | T | 38.6 | 35.8–41.4 |
| | | T/T | 86 | 15.1 | 12.4–18.3 | | | |
| ABCC2 *rs717620* (-24 C > T) | 568 | C/C | 331 | 58.3 | 54.2–62.3 | C | 76.1 | 73.6–78.5 |
| | | C/T | 203 | 35.7 | 31.9–39.8 | T | 23.9 | 21.5–26.4 |
| | | T/T | 34 | 6.0 | 4.3–8.2 | | | |
| ABCG2 *rs2231137* (34 G > A) | 570 | G/G | 504 | 88.4 | 85.5–90.8 | G | 93.9 | 92.4–95.2 |
| | | G/A | 63 | 11.1 | 8.7–13.9 | A | 6.1 | 4.8–7.6 |
| | | A/A | 3 | 0.5 | 0.2–1.5 | | | |
| ABCG2 *rs2231142* (421 C > A) | 569 | C/C | 501 | 88.0 | 85.1–90.5 | C | 93.8 | 92.2–95.0 |
| | | C/A | 65 | 11.4 | 9.1–14.3 | A | 6.2 | 5.0–78.0 |
| | | A/A | 3 | 0.5 | 0.2–1.5 | | | |
| SLCO1A2 *rs11568563* (516 A > C) | 569 | A/A | 494 | 86.8 | 83.8–89.4 | A | 93.4 | 91.8–94.7 |
| | | A/C | 75 | 13.2 | 10.6–16.2 | C | 6.6 | 52.9–81.8 |
| SLCO1A2 *rs11568564* (502 C > T) | 570 | C/C | 568 | 99.6 | 98.7–99.9 | C | 99.8 | 99.4–99.9 |
| | | C/T | 2 | 0.4 | 0.1–1.3 | T | 0.2 | 0.05–0.6 |
| SLCO1B1 *rs2306283* (SLCO1B1*1B) | 568 | A/A | 228 | 40.1 | 36.2–44.2 | A | 62.0 | 59.1–64.8 |
| | | A/G | 248 | 43.7 | 39.6–47.8 | G | 38.0 | 35.2–40.9 |
| | | G/G | 92 | 16.2 | 13.4–19.5 | | | |
| SLCO1B1 *rs4149056* (SLCO1B1*5) | 569 | T/T | 421 | 74.0 | 70.2–77.4 | T | 85.6 | 83.4–87.5 |
| | | T/C | 132 | 23.2 | 19.9–26.8 | C | 14.4 | 12.5–16.6 |
| | | C/C | 16 | 2.8 | 1.7–4.5 | | | |

**TABLE 1.** (*Continued*) Genotype and Allele Frequencies in Drug Transporters in Spanish White Organ Recipients and Donors

| | SNP Database* | | | | | |
|---|---|---|---|---|---|---|
| | Genotype Frequency | | Allele Frequency | | | |
| **Gene SNP** | | **%** | | **%** | ***P* ($\chi^2$, df = 1)** | **Percentage Variation‡** |
| ABCB1 *rs1045642* (3435C > T) | C/C | 15.0 | C | 43.0 | <0.001 | |
| | C/T | 55.8 | T | 57.0 | | −17.5 |
| | T/T | 29.2 | | | | |
| ABCB1 *rs1128503* (1236 T > C) | C/C | 26.5 | C | 54.9 | 0.094 | |
| | C/T | 55.6 | T | 45.1 | | −5.5 |
| | T/T | 16.8 | | | | |
| ABCB1 *rs2032582* (2677 G > T/A) | G/G | 25.7 | G | 53.1 | <0.001 | |
| | G/T | 54.9 | T | 46.9 | | −19.2 |
| | T/T | 19.5 | A | 0.0 | | |
| | G/A | 0.0 | | | | |
| | T/A | 0.0 | | | | |
| | A/A | 0.0 | | | | |
| ABCB1 *rs2229109* (1199 G > A) | G/G | 94.6 | G | 97.3 | <0.001 | |
| | G/A | 5.4 | A | 2.7 | | 114.3 |
| | A/A | 0.0 | | | | |
| ABCB1 *rs2235013* (1725 + 38 G > A) | G/G | 26.8 | G | 55.8 | <0.001 | |
| | A/G | 58.0 | A | 44.2 | | 16.3 |
| | A/A | 15.2 | | | | |
| ABCB1 *rs2235033* (1554 + 24T > C) | T/T | 27.4 | T | 56.2 | <0.001 | |
| | C/T | 57.5 | C | 43.8 | | 11.6 |
| | C/C | 15.0 | | | | |
| ABCB1 *rs3213619* (-129 T > C) | T/T | 93.8 | T | 96.9 | 0.575 | |
| | C/T | 6.2 | C | 3.1 | | −9.3 |
| ABCB1 *rs9282564* (61 A > G) | A/A | 81.7 | A | 90.0 | <0.001 | |
| | A/G | 16.7 | G | 10.0 | | −38.6 |
| | G/G | 1.7 | | | | |
| ABCC2 *rs2273697* (1249 G > A) | G/G | 57.6 | G | 76.3 | 0.001 | |
| | G/A | 37.3 | A | 23.7 | | −18.4 |
| | A/A | 5.1 | | | | |
| ABCC2 *rs3740066* (3972 C > T) | C/C | 40.0 | C | 65.8 | 0.002 | |
| | C/T | 51.7 | T | 34.2 | | 12.8 |
| | T/T | 8.3 | | | | |
| ABCC2 *rs717620* (-24 C > T) | C/C | 56.7 | C | 77.5 | 0.274 | |
| | C/T | 41.7 | T | 22.5 | | 6.0 |
| | T/T | 1.7 | | | | |
| ABCG2 *rs2231137* (34 G > A) | G/G | 96.7 | G | 98.3 | <0.001 | |
| | G/A | 3.3 | A | 1.7 | | 255.7 |
| | A/A | 0.0 | | | | |
| ABCG2 *rs2231142* (421 C > A) | C/C | 78.3 | C | 88.3 | <0.001 | |
| | C/A | 20.0 | A | 11.7 | | −46.7 |
| | A/A | 1.7 | | | | |
| SLCO1A2 *rs11568563* (516 A > C) | A/A | 83.3 | A | 91.7 | 0.037 | |
| | A/C | 16.7 | C | 8.3 | | −56.5 |
| SLCO1A2 *rs11568564* (502 C > T) | C/C | 99.1 | C | 99.6 | 0.230 | |
| | C/T | 0.9 | T | 0.4 | | −20.6 |
| SLCO1B1 *rs2306283* (SLCO1B1*1B) | A/A | 36.3 | A | 59.7 | 0.119 | |
| | A/G | 46.9 | G | 40.3 | | −5.6 |
| | G/G | 16.8 | | | | |
| SLCO1B1 *rs4149056* (SLCO1B1*5) | T/T | 70.0 | T | 84.2 | 0.119 | |
| | T/C | 28.3 | C | 15.8 | | −8.8 |
| | C/C | 1.7 | | | | |

*Genotype and allele frequencies from SNP database (www.ncbi.nlm.nih.gov/projects/SNP/) correspond to HapMap CEU (European).
†Percentage variation in variant allele frequency (%; a positive value indicates an increase with respect to values described in dbSNP, whereas a negative value represents a decline).
95% CI, 95% confidence interval; df, degrees of freedom; n, number of samples correctly genotyped.

**TABLE 2.** Genotype and Allele Frequencies in Drug-Metabolizing Enzymes in Spanish White Organ Recipients and Donors

| | | Spanish White Population | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Genotype Frequency | | | | Allele Frequency | | |
| Gene SNP | n | | n | % | 95% CI | | % | 95% CI |
| CYP2B6 *rs2279343* (CYP2B6*4) | 569 | A/A | 279 | 49.0 | 44.9–53.1 | A | 69.4 | 66.7–72.0 |
| | | A/G | 232 | 40.8 | 36.8–44.9 | G | 30.6 | 28.0–33.3 |
| | | G/G | 58 | 10.2 | 8.0–13.0 | | | |
| CYP2C9 *rs1057910* (CYP2C9*3) | 549 | A/A | 492 | 89.6 | 86.8–91.9 | A | 94.5 | 93.0–95.7 |
| | | A/C | 54 | 9.8 | 7.6–12.6 | C | 5.5 | 4.3–7.0 |
| | | C/C | 3 | 0.5 | 0.2–1.6 | | | |
| CYP2C19 *rs4244285* (CYP2C19*2) | 570 | G/G | 420 | 73.7 | 69.9–77.1 | G | 85.1 | 82.9–87.0 |
| | | G/A | 130 | 22.8 | 19.6–26.4 | A | 14.9 | 13.0–17.1 |
| | | A/A | 20 | 3.5 | 2.3–5.4 | | | |
| CYP3A4 *rs2740574* (CYP3A4*1B) | 570 | A/A | 530 | 93.0 | 90.6–94.8 | A | 96.3 | 95.1–97.3 |
| | | A/G | 38 | 6.7 | 4.9–9.0 | G | 3.7 | 2.7–5.0 |
| | | G/G | 2 | 0.4 | 0.1–1.3 | | | |
| CYP3A5 *rs776746* (CYP3A5*3) | 568 | A/A | 6 | 1.1 | 0.5–2.3 | A | 8.9 | 7.4–10.7 |
| | | A/G | 89 | 15.7 | 12.9–18.9 | G | 91.1 | 89.3–92.6 |
| | | G/G | 473 | 83.3 | 80.0–86.1 | | | |
| CYP3A5 *rs41303343* (CYP3A5*7) | 570 | -/- | 566 | 99.3 | 98.2–98.2 | — | 99.6 | 99.1–99.7 |
| | | -/T | 4 | 0.7 | 0.3–1.8 | T | 0.4 | 0.1–0.9 |
| CYP3A5 *rs10264272* (CYP3A5*6) | 570 | G/G | 565 | 99.1 | 98.0–99.6 | G | 99.6 | 99.0–99.8 |
| | | G/A | 5 | 0.9 | 0.4–2.0 | A | 0.4 | 0.2–1.0 |
| MTHFR *rs1801131* (1298A > C) | 562 | A/A | 328 | 58.4 | 54.2–62.4 | A | 74.5 | 71.8–76.9 |
| | | A/C | 181 | 32.2 | 28.5–36.2 | C | 25.5 | 23.1–28.2 |
| | | C/C | 53 | 9.4 | 7.3–12.1 | | | |
| MTHFR *rs1801133* (677 C > T) | 569 | C/C | 191 | 33.6 | 29.8–37.5 | C | 59.2 | 56.3–62.0 |
| | | C/T | 292 | 51.3 | 47.2–55.4 | T | 40.8 | 38.8–43.6 |
| | | T/T | 86 | 15.1 | 12.4–18.3 | | | |
| NOD2/CARD15 *rs2066844* (R702W) | 570 | C/C | 500 | 87.7 | 84.8–90.2 | C | 93.6 | 92.0–94.9 |
| | | C/T | 67 | 11.8 | 9.4–14.7 | T | 6.4 | 5.1–8.0 |
| | | T/T | 3 | 0.5 | 0.2–1.5 | | | |
| NOD2/CARD15 *rs2066845* (G908R) | 569 | G/G | 551 | 96.8 | 95.1–98.0 | G | 98.4 | 97.5–99.9 |
| | | G/C | 18 | 3.2 | 2.0–4.9 | C | 1.6 | 1.0–2.5 |
| TPMT *rs1142345* (TPMT*3C) | 569 | A/A | 525 | 92.3 | 89.8–94.2 | A | 96.0 | 94.9–97.2 |
| | | A/G | 43 | 7.6 | 5.7–10.0 | G | 4.0 | 28.2–50.6 |
| | | G/G | 1 | 0.2 | 0.0–1.0 | | | |
| TPMT *rs1800460* (TPMT*3B) | 569 | G/G | 530 | 93.1 | 90.8–94.9 | G | 96.6 | 95.3–97.5 |
| | | G/A | 39 | 6.9 | 5.1–9.2 | A | 3.4 | 2.5–4.6 |
| TPMT *rs1800462* (TPMT*2) | 569 | G/G | 562 | 98.8 | 97.5–99.4 | G | 99.4 | 98.7–99.7 |
| | | G/C | 7 | 1.2 | 0.6–2.5 | C | 0.6 | 0.3–1.7 |
| UGT1A9 *rs72551330* (98T > C) | 570 | T/T | 535 | 93.9 | 91.6–95.6 | T | 96.9 | 95.8–97.8 |
| | | T/C | 35 | 6.1 | 4.4–8.4 | C | 3.1 | 2.2–4.2 |
| UGT1A9 *rs6714486* (−275T > A) | 566 | T/T | 500 | 88.3 | 85.4–90.7 | T | 94.2 | 92.7–95.5 |
| | | T/A | 65 | 11.5 | 9.1–14.4 | A | 5.8 | 4.5–7.3 |
| | | A/A | 1 | 0.2 | 0.0–1.0 | | | |

| | SNP Database* | | | | | |
|---|---|---|---|---|---|---|
| | Genotype Frequency | | Allele Frequency | | | |
| Gene SNP | | % | | % | P ($\chi^2$, df = 1) | Percentage Variation† |
| CYP2B6 *rs2279343* (CYP2B6*4) | A/A | 61.9 | A | 78.6‡ | <0.001 | |
| | A/G | 33.3 | G | 21.4 | | 42.9% |
| | G/G | 4.8 | | | | |
| CYP2C9 *rs1057910* (CYP2C9*3) | A/A | 88.3 | A | 94.2 | 0.634 | |
| | A/C | 11.7 | C | 5.8 | | −5.8% |
| | C/C | 0.0 | | | | |

**TABLE 2.** (*Continued*) Genotype and Allele Frequencies in Drug-Metabolizing Enzymes in Spanish White Organ Recipients and Donors

| | SNP Database* | | | | | |
|---|---|---|---|---|---|---|
| | Genotype Frequency | | Allele Frequency | | | |
| Gene SNP | | % | | % | P ($\chi^2$, df = 1) | Percentage Variation† |
| CYP2C19 *rs4244285* (CYP2C19*2) | G/G | 74.1 | G | 84.5 | 0.583 | |
| | G/A | 20.7 | A | 15.5 | | −3.8% |
| | A/A | 5.2 | | | | |
| CYP3A4 *rs2740574* (CYP3A4*1B) | A/A | 95.5 | A | 97.7‡ | 0.002 | |
| | A/G | 4.5 | G | 2.3 | | 60.3% |
| | G/G | 0.0 | | | | |
| CYP3A5 *rs776746* (CYP3A5*3) | A/A | 0.0 | A | 3.6 | <0.001 | |
| | A/G | 7.2 | G | 96.4 | | 146.9% |
| | G/G | 92.8 | | | | |
| CYP3A5 *rs41303343* (CYP3A5*7) | −/− | 100.0 | — | 100.0‡ | <0.001 | |
| | -/T | 0.0 | T | 0.0 | | — |
| CYP3A5 *rs10264272* (CYP3A5*6) | G/G | 100.0 | G | 100.0 | <0.001 | |
| | G/A | 0.0 | A | 0.0 | | — |
| MTHFR *rs1801131* (1298A > C) | A/A | 43.4 | A | 65.9 | <0.001 | |
| | A/C | 45.1 | C | 34.1 | | −25.1% |
| | C/C | 11.5 | | | | |
| MTHFR *rs1801133* (677 C > T) | C/C | 46.9 | C | 69.0 | <0.001 | |
| | C/T | 44.2 | T | 31.0 | | 31.5% |
| | T/T | 8.8 | | | | |
| NOD2/CARD15 *rs2066844* (R702W) | C/C | 95.8 | C | 97.9§ | <0.001 | |
| | C/T | 4.2 | T | 2.1 | | 205.4% |
| | T/T | 0.0 | | | | |
| NOD2/CARD15 *rs2066845* (G908R) | G/G | 91.3 | G | 95.7§ | <0.001 | |
| | G/C | 8.7 | C | 4.3 | | −52.1% |
| TPMT *rs1142345* (TPMT*3C) | A/A | 94.7 | A | 97.3 | 0.024 | |
| | A/G | 5.3 | G | 2.7 | | 40.2% |
| | G/G | 0.0 | | | | |
| TPMT *rs1800460* (TPMT*3B) | G/G | 94.8 | G | 97.4¶ | 0.080 | |
| | G/A | 5.2 | A | 2.6 | | 31.8% |
| TPMT *rs1800462* (TPMT*2) | G/G | 99.7 | G | 99.8# | 0.002 | |
| | G/C | 0.3 | C | 0.2 | | 204.3% |
| UGT1A9 *rs72551330* (98T > C) | T/T | 98.2 | T | 99.1# | <0.001 | |
| | T/C | 1.2 | C | 0.9 | | 239.8% |
| UGT1A9 *rs6714486* (−275T > A) | T/T | 85.0 | T | 92.5 | 0.026 | |
| | T/A | 15.0 | A | 7.5 | | −23.3% |
| | A/A | 0.0 | | | | |

*Genotype and allele frequencies from SNP database (www.ncbi.nlm.nih.gov/projects/SNP/) correspond to HapMap CEU (European). Other white populations are indicated only when HapMap data are not available.

†Percentage variation in variant allele frequency (%; a positive value indicates an increase with respect to values described in dbSNP, whereas a negative value represents a decline).

‡EGP_CEPH-PANEL (European).

§AFD_EUR_PANEL (European).

¶CAUC2.

#ESP_Cohort_Populations.

95% CI, 95% confidence interval; df, degrees of freedom; n, number of samples correctly genotyped.

higher for wild-type patients, but this has not been confirmed by other studies.[10,20–22] ABCB1 3435C > T has also been associated with methotrexate clearance[23] and response in rheumatoid arthritis.[24] The genotype distribution and variant T allele frequency in 3435C > T (Table 1), although different to other white populations, were similar to those previously reported in the Spanish population.[14,25]

CYP3A5 is responsible for tacrolimus metabolism and, although the presence of CYP3A5*1/*1 among white populations is extremely rare, 1.1% of our population presented this genotype. CYP3A5*1/*1 and *1/*3 variants are associated with increased metabolism of tacrolimus and dose requirements compared with patients carrying the *3/*3 genotype[2,11,21,26,27] and also with an increased risk of rejection

**TABLE 3.** CYP3A4 *rs2740574* (CYP3A4*1B) and CYP3A5 *rs776746* (CYP3A5*3) Genotypes

| | | Genotype Frequency | | |
|---|---|---|---|---|
| **CYP3A4 *rs2740574*** | **CYP3A5 *rs776746*** | **n** | **%** | **95% CI** |
| 1 A/1A | *3/*3 | 465 | 81.9 | 78.5–84.8 |
| 1 A/1A | *1/*3 | 60 | 10.6 | 8.3–13.4 |
| 1A/1A | *1/*1 | 3 | 0.5 | 0.18–1.5 |
| 1A/1B | *3/*3 | 8 | 1.4 | 0.7–2.8 |
| 1A/1B | *1/*3 | 28 | 4.9 | 3.4–7.0 |
| 1A/1B | *1/*1 | 2 | 0.4 | 0.1–1.3 |
| 1B/1B | *1/*1 | 2 | 0.4 | 0.1–1.3 |
| Total | | 568 | 100.0 | |

in kidney transplantation.[28] The increased presence of the *1/*1 genotype in the Spanish white population has been reported previously.[14,29] This could mean that the initial tacrolimus dose requirements may be higher in the Spanish population.

Although no CYP3A4*1B homozygote variant individuals were reported in dbSNP,[15,16] a small proportion was found in our population, and the variant allele frequency was higher but similar to that reported previously in other Spanish populations (3.7%–4.8%).[29] This variant has been associated with higher dose requirements of tacrolimus and cyclosporine[21,30] and increased clearance of docetaxel.[31] Also, all subjects carrying the CYP3A4*1B variant also carried the CYP3A5*1 allele and most of the patients heterozygous for CYP3A4*1B also carried CYP3A5*1/*3. These genotype frequencies were very similar to those described previously for the Spanish population,[29] though their clinical consequences are not clear.

Two SNPs in the MTHFR gene, 677C > T and 1298A > C, have been associated with reduced MTHFR enzyme activity and methotrexate-related toxicity.[32] A higher frequency than reported in dbSNP of T allele in 677C > T was found. This frequency was similar to those reported by other authors for the Spanish population (37.0%–39.0%)[33] and to those reported for other white populations (31.0%–34.0%).[34,35] Patients carrying the variant T/T genotype have an increased risk of adverse events and protective effect on acute graft versus host disease.[24,36,37] This fact points out the importance of MTHFR genotyping in the Spanish population for the prevention of adverse events.

The presence of 1 or 2 inactive TPMT alleles (*2, *3A, *3B, *3C, and *4) is a risk factor for severe myelosuppression due to thiopurines.[38] A higher frequency of variant allele was found for *2 (*rs1800462*), *3B (*rs1800460*), and *3C (*rs1142345*). These frequencies were also higher than those reported for other white populations (*2, 0.2%–0.7%; and *3C, 0.4%–1.3%).[39,40] Dosage recommendations for thiopurines regarding TPMT genotype have been published,[13,41] and important dose reductions are required in these patients. Thus, the Spanish population would especially benefit from genotyping before starting treatment.

Variant allele frequencies for UGT1A9 98T > C-275T > A, which are SNPs that have been associated with altered pharmacokinetics of MPA,[42] were consistent with those previously reported for white populations (2.2%–3.6%[43] and 4.0%–8.0%,[44] respectively), but different from those described in dbSNP.

ABCC2-24C > T, 1249 G > A, and ABCG2 421C > A are associated with altered pharmacokinetics and increased toxicity of MPA[8,45–47] and methotrexate.[48,49] The proportion of patients carrying the variant allele in ABCC2-24C > T and ABCC2 1249 G > A was similar to those reported by other authors for white populations[45,50] and, for ABCG2 421C > A, lower than in HapMap CEU but similar to HapMap TSI (Tuscan, Italy) (7.4%).[15,16]

No differences in variant allele frequencies were found for CYP2C9*3 (associated with decreased enzymatic activity and different warfarin dosage[51]), CYP2C19*2 (therapeutic recommendations regarding CYP2C19 genotype have been published for clopidogrel,[41,52] citalopram, imipramine, and voriconazole[41]), SLCO1A2 516A > C and 502C > T (associated with reduced uptake of methotrexate[53] and different response), and SLCO1B1 *rs4149056* (SNP associated with altered pharmacokinetics of MPA[8,47,54] and methotrexate[9,48,55] and which also markedly increases systemic exposure to simvastatin and the risk of muscle toxicity—so dosage recommendations of simvastatin have been published[56]).

In summary, different allele frequencies have been found between the Spanish white population and other whites in variants such as CYP3A5*3, ABCB1 3435C > T, MTHFR 677C > T, and 1298A > C, TPMT * 2, * 3A, * 3B, and * 3C, for which there is abundant evidence of clinical implications. The importance of these different allele frequencies is difficult to interpret, and their clinical significance is therefore still unclear. However, variant allele frequency is very important to establish the cost-effectiveness of a particular pharmacogenetic test and, consequently, for deciding whether or not to perform it on a routine basis in a given population once the genotype–phenotype relationship and the clinical significance of the variation have been well established.

## CONCLUSIONS

In conclusion, in this Spanish population, the genotype and allele frequencies of 33 SNPs in 14 different genes encoding for drug-metabolizing enzymes and drug transporters were determined, showing differences between the Spanish white population and other white populations in allele frequency and genotypic distribution. Such differences may have implications in relation to dose requirements and drug-induced toxicity. These data are important for further research to help explain interindividual pharmacokinetic and pharmacodynamic variability in response to drug therapy.

## REFERENCES

1. Whirl-Carrillo M, McDonagh EM, Hebert JM, et al. Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther*. 2012;92:414–417.
2. De Jonge H, Kuypers DRJ. Pharmacogenetics in solid organ transplantation: current status and future directions. *Transplant Rev (Orlando)*. 2008;22:6–20.

 

3. Johnson AD, Wang D, Sadee W. Polymorphisms affecting gene regulation and mRNA processing: broad implications for pharmacogenetics. *Pharmacol Ther.* 2005;106:19–38.
4. Herrero Cervera M, Aliño Pellicer S, Poveda Andrés J, et al. *Farmacogenética: una realidad clínica*. 1st ed. Valencia, Spain: Master Line; 2010.
5. Herrero MJ, Almenar L, Jordán C, et al. Clinical interest of pharmacogenetic polymorphisms in the immunosuppressive treatment after heart transplantation. *Transplant Proc.* 2010;42:3181–3182.
6. Herrero MJ, Sánchez-Plumed J, Galiana M, et al. Influence of pharmacogenetic polymorphisms in routine immunosuppression therapy after renal transplantation. *Transplant Proc.* 2010;42:3134–3136.
7. Jordán de Luna C, Herrero Cervera MJ, Sánchez Lázaro I, et al. Pharmacogenetic study of ABCB1 and CYP3A5 genes during the first year following heart transplantation regarding tacrolimus or cyclosporine levels. *Transplant Proc.* 2011;43:2241–2243.
8. Miura M, Satoh S, Inoue K, et al. Influence of SLCO1B1, 1B3, 2B1 and ABCC2 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. *Eur J Clin Pharmacol.* 2007;63:1161–1169.
9. Ramsey LB, Bruun GH, Yang W, et al. Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. *Genome Res.* 2012;22:1–8.
10. Haufroid V, Mourad M, Van Kerckhove V, et al. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics*. 2004;14:147–154.
11. Thervet E, Loriot M, Barbier S, et al. Optimization of initial tacrolimus dose using pharmacogenetic testing. *Clin Pharmacol Ther.* 2010;87:721–726.
12. Fisher MC, Cronstein BN. Metaanalysis of methylenetetrahydrofolate reductase (MTHFR) polymorphisms affecting methotrexate toxicity. *J Rheumatol.* 2009;36:539–545.
13. Relling M V, Gardner EE, Sandborn WJ, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. *Clin Pharmacol Ther.* 2011;89:387–391.
14. Gervasini G, Vizcaino S, Gasiba C, et al. Differences in CYP3A5*3 genotype distribution and combinations with other polymorphisms between Spaniards and other Caucasian populations. *Ther Drug Monit.* 2005;27:819–821.
15. NCBI. Database of single nucleotide polymorphisms (dbSNP). Available at: www.ncbi.nlm.nih.gov/projects/SNP/. Accessed October 2012.
16. International HapMap Project. Available at: http://hapmap.ncbi.nlm.nih.gov/. Accessed October 2012.
17. Zheng H, Schuetz E, Zeevi A, et al. Sequential analysis of tacrolimus dosing in adult lung transplant patients with ABCB1 haplotypes. *J Clin Pharmacol.* 2005;45:404–410.
18. Anglicheau D, Verstuyft C, Laurent-Puig P, et al. Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. *J Am Soc Nephrol.* 2003;14:1889–1896.
19. Yates CR, Zhang W, Song P, et al. The effect of CYP3A5 and MDR1 polymorphic expression on cyclosporine oral disposition in renal transplant patients. *J Clin Pharmacol.* 2003;43:555–564.
20. Tsuchiya N, Satoh S, Tada H, et al. Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. *Transplantation*. 2004;78:1182–1187.
21. Hesselink DA, Van Schaik RHN, Van der Heiden IP, et al. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther.* 2003;74:245–254.
22. Bouamar R, Hesselink DA, Van Schaik R, et al. Polymorphisms in CYP3A5, CYP3A4, and ABCB1 are not associated with cyclosporine pharmacokinetics nor with cyclosporine clinical end points after renal transplantation. *Ther Drug Monit.* 2011;33:178–184.
23. Kim IW, Yun HY, Choi B, et al. ABCB1 C3435T genetic polymorphism on population pharmacokinetics of methotrexate after hematopoietic stem cell transplantation in Korean patients: a prospective analysis. *Clin Ther.* 2012;34:1816–1826.
24. Kato T, Hamada A, Mori S, et al. Genetic polymorphisms in metabolic and cellular transport pathway of methotrexate impact clinical outcome of methotrexate monotherapy in Japanese patients with rheumatoid arthritis. *Drug Metab Pharmacokinet.* 2012;27:192–199.
25. Bernal ML, Sinues B, Fanlo A, et al. Frequency distribution of C3435T mutation in exon 26 of the MDR1 gene in a Spanish population. *Ther Drug Monit.* 2003;25:107–111.
26. Tang H, Xie H, Yao Y, et al. Lower tacrolimus daily dose requirements and acute rejection rates in the CYP3A5 nonexpressers than expressers. *Pharmacogenet Genomics.* 2011;21:713–720.
27. Santoro A, Felipe CR, Tedesco-Silva H, et al. Pharmacogenetics of calcineurin inhibitors in Brazilian renal transplant patients. *Pharmacogenomics*. 2011;12:1293–1303.
28. Min S-I, Kim SY, Ahn SH, et al. CYP3A5 *1 allele: impacts on early acute rejection and graft function in tacrolimus-based renal transplant recipients. *Transplantation*. 2010;90:1394–1400.
29. Gervasini G, García-Martín E, Ladero JM, et al. Genetic variability in CYP3A4 and CYP3A5 in primary liver, gastric and colorectal cancer patients. *BMC Cancer*. 2007;7:118.
30. Kuypers DRJ, De Jonge H, Naesens M, et al. CYP3A5 and CYP3A4 but not MDR1 single-nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. *Clin Pharmacol Ther.* 2007;82:711–725.
31. Baker SD, Verweij J, Cusatis GA, et al. Pharmacogenetic pathway analysis of docetaxel elimination. *Clin Pharmacol Ther.* 2009;85:155–163.
32. Hughes LB, Beasley TM, Patel H, et al. Racial or ethnic differences in allele frequencies of single-nucleotide polymorphisms in the methylenetetrahydrofolate reductase gene and their influence on response to methotrexate in rheumatoid arthritis. *Ann Rheum Dis.* 2006;65:1213–1218.
33. Martínez-Frías ML, Bermejo E, Pérez B, et al. Analysis of the frequencies of genotype combinations of 4 polymorphisms of genes acting on the folate cycle in the Spanish population [in Spanish]. *Med Clin (Barc).* 2008;131:81–88.
34. Kurzwelly D, Knop S, Guenther M, et al. Genetic variants of folate and methionine metabolism and PCNSL incidence in a German patient population. *J Neurooncol.* 2010;100:187–192.
35. Linnebank M, Homberger A, Nowak-Göttl U, et al. Linkage disequilibrium of the common mutations 677C > T and 1298A > C of the human methylenetetrahydrofolate reductase gene as proven by the novel polymorphisms 129C > T, 1068C > T. *Eur J Pediatr.* 2000;159:472–473.
36. Robien K, Schubert MM, Chay T, et al. Methylenetetrahydrofolate reductase and thymidylate synthase genotypes modify oral mucositis severity following hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2006;37:799–800.
37. Spyridopoulou KP, Dimou NL, Hamodrakas SJ, et al. Methylene tetrahydrofolate reductase gene polymorphisms and their association with methotrexate toxicity: a meta-analysis. *Pharmacogenet Genomics.* 2012;22:117–133.
38. Nguyen CM, Mendes MAS, Ma JD. Thiopurine methyltransferase (TPMT) genotyping to predict myelosuppression risk. *PLoS Curr.* 2011;3:RRN1236.
39. Schaeffeler E, Fischer C, Brockmeier D, et al. Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. *Pharmacogenetics*. 2004;14:407–417.
40. Spire-Vayron de la Moureyre C, Debuysere H, Mastain B, et al. Genotypic and phenotypic analysis of the polymorphic thiopurine S-methyltransferase gene (TPMT) in a European population. *Br J Pharmacol.* 1998;125:879–887.
41. Swen JJ, Nijenhuis M, De Boer A, et al. Pharmacogenetics: from bench to byte—an update of guidelines. *Clin Pharmacol Ther.* 2011;89:662–673.
42. Kuypers DRJ, Naesens M, Vermeire S, et al. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in de novo renal allograft recipients *Clin Pharmacol Ther.* 2005;78:351–361.
43. Villeneuve L, Girard H, Fortier L-C, et al. Novel functional polymorphisms in the UGT1A7 and UGT1A9 glucuronidating enzymes in Caucasian and African-American subjects and their impact on the metabolism of 7-ethyl-10-hydroxycamptothecin and flavopiridol anticancer drugs. *J Pharmacol Exp Ther.* 2003;307:117–128.
44. Ramírez J, Liu W, Mirkov S, et al. Lack of association between common polymorphisms in UGT1A9 and gene expression and activity. *Drug Metab Dispos.* 2007;35:2149–2153.

45. Naesens M, Kuypers DRJ, Verbeke K, et al. Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. *Transplantation*. 2006;82:1074–1084.
46. Geng F, Jiao Z, Dao YJ, et al. The association of the UGT1A8, SLCO1B3 and ABCC2/ABCG2 genetic polymorphisms with the pharmacokinetics of mycophenolic acid and its phenolic glucuronide metabolite in Chinese individuals. *Clin Chim Acta*. 2012;413:683–690.
47. Miura M, Kagaya H, Satoh S, et al. Influence of drug transporters and UGT polymorphisms on pharmacokinetics of phenolic glucuronide metabolite of mycophenolic acid in Japanese renal transplant recipients. *Ther Drug Monit*. 2008;30:559–564.
48. Lopez-Lopez E, Martin-Guerrero I, Ballesteros J, et al. Polymorphisms of the SLCO1B1 gene predict methotrexate-related toxicity in childhood acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2011;57:612–619.
49. Rau T, Erney B, Göres R, et al. High-dose methotrexate in pediatric acute lymphoblastic leukemia: impact of ABCC2 polymorphisms on plasma concentrations. *Clin Pharmacol Ther*. 2006;80:468–476.
50. Daly AK, Aithal GP, Leathart JBS, et al. Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABCC2 genotypes. *Gastroenterology*. 2007;132:272–281.
51. Chan SL, Suo C, Lee SC, et al. Translational aspects of genetic factors in the prediction of drug response variability: a case study of warfarin pharmacogenomics in a multi-ethnic cohort from Asia. *Pharmacogenomics J*. 2012;12:312–318.
52. Scott S, Sangkuhl K, Gardner EE, et al. Clinical pharmacogenetics implementation consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. *Clin Pharmacol Ther*. 2011;90:328–332.
53. Badagnani I, Castro RA, Taylor TR, et al. Interaction of methotrexate with organic-anion transporting polypeptide 1A2 and its genetic variants. *J Pharmacol Exp Ther*. 2006;318:521–529.
54. Michelon H, König J, Durrbach A, et al. SLCO1B1 genetic polymorphism influences mycophenolic acid tolerance in renal transplant recipients. *Pharmacogenomics*. 2010;11:1703–1713.
55. Treviño LR, Shimasaki N, Yang W, et al. Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. *J Clin Oncol*. 2009;27:5972–5978.
56. Wilke R, Ramsey LB, Johnson SG, et al. The clinical pharmacogenomics implementation consortium: CPIC guideline for SLCO1B1 and simvastatin-induced myopathy. *Clin Pharmacol Ther*. 2012;92:112–117.