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Genetic Polymorphisms in *CYP3A5* and *MDR1* Genes and Their Correlations With Plasma Levels of Tacrolimus and Cyclosporine in Renal Transplant Recipients

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ABSTRACT

Immunosuppressive drugs, such as tacrolimus (FK506) and cyclosporine (CsA), play an essential role in graft survival, preventing rejection. Large interindividual differences in drug-metabolizing enzymes as well as in drug transporters make the task of reaching the optimal concentrations difficult. The bioavailability of CsA and FK506 seems to be associated with the cytocrhome P450 IIIA (*CYP3A*) gene. It has also been described that the Multi Drug Resistance 1 (*MDR1*) gene that encodes for polyglycoprotein-P (P-gp) may influence the metabolizing action of FK506 and CsA. Therefore, we sought, to correlate single nucleotide polymorphisms (SNPs) in the *CYP3A* and *MDR1* genes with the concentrations of FK506 and CsA. For this purpose we analyzed 2 groups of renal transplant recipients by sequencing: one receiving a CsA immunosuppressive regime, and other, an FK506-immunosuppression. This study showed that subjects in the FK506 group who had encoded the 1236C>T substitution in the *MDR1* gene displayed 44.4% higher drug concentrations compared with ("wild-type") individuals. Individuals carrying the 2677G>T,A mutation showed FK506 concentrations that were 44.7% higher than the wild-type individuals. Concerning the CsA group, individuals carrying the 22915A>C substitution displayed CsA concentrations 52.1% higher than wild-type individuals.

PHARMACOGENETICS applies information related to genetic variability in relation to pharmacodynamics and metabolism of drugs. Some variations, referred to as single nucleotide polymorphisms (SNPs), when present in drug-metabolizing enzymes or drug transporters, may significantly alter the dosing of a given drug. Cytochrome P450 IIIA (*CYP3A*), and in particular its variant *CYP3A5*, plays a key role in metabolism of immunosuppressive drugs, such has tacrolimus (FK506) and cyclosporine (CsA), as well as a great variety of other substrates.[1] Also, the drug transporter encoded by the Multi Drug Resistance (*MDR1*) gene, P-glycoprotein, which is responsible for actively exporting substrates from the inside of the cell to the exterior environment,[2] plays a role. Several authors have described FK506 and CsA to be substrates for P-glycoprotein, making the genetic characterization of this gene of great relevance.[1] In this study, we have focused on the third exon of the *CYP3A5* gene. Polymorphisms in this region have been reported to have a relation to altered FK506 requirements.[3,4] As regards the *MDR1* gene, we analyzed a 2677G>T,A substitution in exon 21 and a 1236C>T substitution in exon 12, both of which other studies have shown to significantly contribute to altered concentrations of immunosuppressants.[5]

MATERIALS AND METHODS

Peripheral blood samples were collected from 2 groups of patients who underwent cadaveric renal transplantation. One group of 30 individuals (13 females and 17 males) received CsA-based immunosuppression regime; another 30 individuals (18 females and 12 males), FK506.

DNA was extracted using MagAttract DNA Blood Midi M48 Kit (Qiagen, Valencia, Calif). Amplification and sequencing primers

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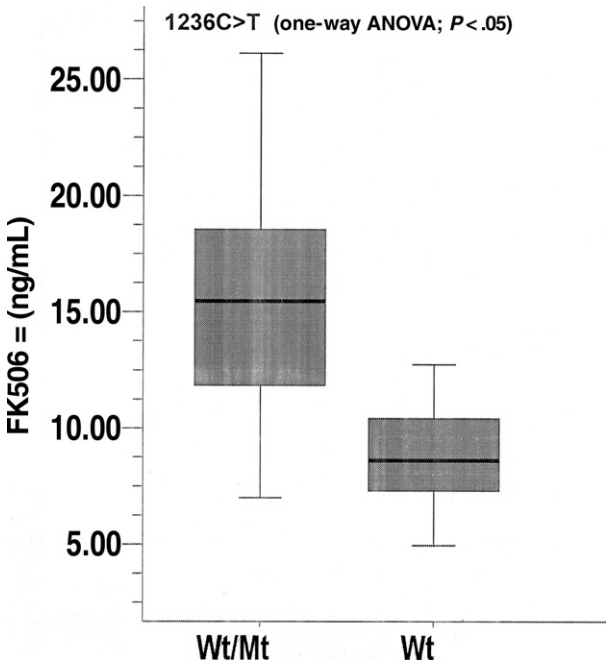

Fig 1. Correlation between levels of FK506 (ng/mL) and the SNP 1236C>T in exon 12 of the *MDR1* gene. Concentration distribution in accordance with the allelic variation. Wt, wild-type genotype; Mt, mutant genotype.

were obtained from Invitrogen. For genotype determination of exon 3 in the *CYP3A5* gene, a forward 3′-CTTAACGAATGCTC-TACTGTC-5′ primer was used, along with the reverse 3′-ACAC-CCAGGAAGCCAGAC-5′ primer. Regarding the *MDR1* gene, genotyping of exon 12 was achieved with a forward primer 3′-TCCTGTGTCTGTGAATTGCCTTG-5′ and reverse 3′-GCT-GATCACCGCAGTCTAGCTCGC-5′. Exon 21 of the *MDR1* gene was genotyped with the forward 3′-GCAGGCTATAGGTTC-CAGGCT-5′ primer and with the reverse 3′-AACAGCCGGGT-GGTGTCA-5′ primer. Amplification polymerase chain reaction (PCR) was performed in a final volume of 50 μL using 60 ng of genomic DNA with 10 pmol of each primer and 1.75 U of Taq polymerase (Promega, Madison, Wisc). PCR products were sequenced with BigDye Terminator V.1.1. (Applied Biosystems, Foster City, Calif) in an ABI PRISM Genetic Analyzer (Applied Biosystems). The SNP analysis resultant from sequencing was correlated with the concentrations of FK506 and CsA using a one-way ANOVA test. Considering that the initial dose was given as 0.15 mg/kg for FK506 and 8 mg/kg for CsA, the first measurement of C_0 (ng/mL), after intravenous administration, was taken in account.

RESULTS

After SNP genotype analysis, we observed that the CsA group showed a 50% frequency of the SNP 22915A>C in exon 3 of the *CYP3A5* gene (n = 26). In the *MDR1* gene, the SNP 1236C>T occurred with a 40.74% frequency rate (n = 27) and for the SNP 2677G>T,A, the frequency was 31.82% (n = 22). For the FK506 group, the SNP in the MDR1 gene 1236C>T occurred at a 34.78% (n = 23) rate, and the 2677G>T,A substitution, 38.89% (n = 18). The correlation of these data with concentrations of FK506 and CsA revealed that those receiving FK506 and were heterozygous for the SNP 1236C>T showed concentrations 44.4% higher ($P < .05$) than those of wild-type individuals (Fig 1). For the same group of individuals, those heterozygous for the 2677T>A substitution, showed FK506 44.7% higher ($P < .05$) values than wild-type individuals (Fig 2). For the CsA group, we observed that the 22915A>C substitution contributed a 52.1% higher concentration of CsA than wild-type individuals (Fig 3).

DISCUSSION

The characterization of genes with a relation to the pharmacodynamics and metabolism of drugs displaying a narrow therapeutic index, such as immunosuppressants, is of great use to clinicians. In this manner, and with the growing knowledge of a vast number of SNPs associated with metabolizing enzymes, as well as with drug transporters, it may be possible to determine an individual's genetic profile for a metabolizing capacity related to a specific drug. In this study, we demonstrated that individuals heterozygous for the 1236C>T and 2677G>T,A SNPs in the *MDR1* gene show less efficient metabolism concerning FK506 compared with those displaying a wild-type genotype. Thus ideally they need lesser amounts of a given drug to reach the same concentrations. The same consideration is possible to establish for individuals heterozygous for the 22915A>C SNP in the *CYP3A5* gene, in relation to the use of CsA. The pharmacogenetic approach to metabolism represents an

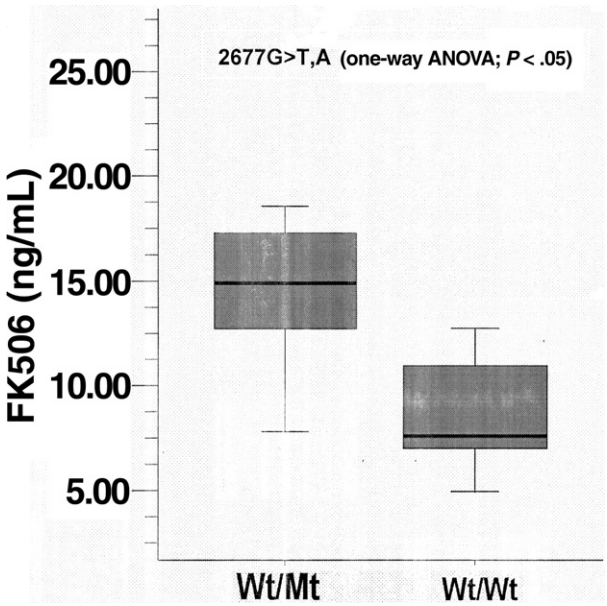

Fig 2. Correlation between plasma levels of FK506 (ng/mL) and the SNP 2277G>T,A in exon 21 of the *MDR1* gene. Concentration distribution in accordance with the allelic variation. Wt, wild-type genotype; Mt, mutant genotype.

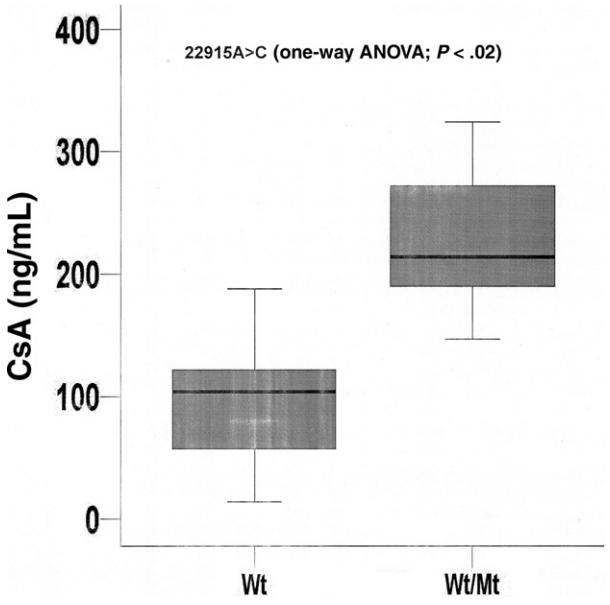

Fig 3. Correlation between the plasma levels of CsA (ng/mL) and the SNP 22915A>C in exon 3 of the *CYP3A5* gene. Concentration distribution in accordance with the allelic variation. Wt, wild-type genotype; Mt, mutant genotype.

alternative to pharmacokinetic studies conducted posttransplantation, in which several blood samples must be obtained at regular intervals to determine the ideal dose to reach the optimal concentrations.

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