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Pharmacogenetics of Immunosuppressive Drugs in Renal Transplantation

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1. Introduction

Facing the demand of obtaining the best cost-efficacy treatments, we are always searching for new alternatives to the existing therapies. The incorporation of new diagnostic techniques and screening, along with the continued development of safer and more effective drugs has improved considerably the expectations and the quality of life of patients.

Still, there is no ideal solution to many of the diseases faced by health professionals in daily clinical practice, so we still have to look for alternatives to the established treatments. The idea of a targeted and personalized therapy to achieve therapeutic success is a goal that is getting more and more important every day. In this context there arises the concept of personalized medicine which is related in our case to the genetic variability associated with different individual response to the same treatment. That is, there is a difference in the response to the same drug in different patients that appears to be related to the different versions of each patient's genes coding for transport proteins, for enzymes involved in metabolism and those genes responsible for the drug mechanism of action, all necessary for the drug to perform its therapeutic effect. This kind of research is developed by two disciplines: pharmacogenetics and pharmacogenomics. These two terms are often mixed and difficult to distinguish, so the international regulatory organizations have tried to fix the proper definitions of both terms. The European Medicines Agency shows the definitions on its web site (www.ema.europa.eu, EMEA/CHMP/ICH/437986/2006) where according to the the International Conference on Harmonisation (ICH), Pharmacogenomics is defined as the study of variations of DNA and RNA characteristics as related to drug response, while Pharmacogenetics is a subset of pharmacogenomics and is defined as the study of variations in DNA sequence as related to drug response.

So, in practice Pharmacogenetics studies the influence of genetic factors in transport, metabolism and drug action. The examples of pharmacogenetic tests employed in the clinical practice are increasing day by day, as for instance when establishing first treatment

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in HIV patients, before employing Cetuximab in colorectal cancer and before Warfarin treatment. In these cases, where it has been demonstrated that it exists a correlation between some known genetic variants and low or no response to treatment, or even occurrence of adverse effects, the clinician needs the genetic test tool in order to settle the correct therapy to each patient. The increase of this genetic testing need is reflected also by the United States Food and Drug Administration (FDA) that publishes on its web site the list containing those drugs with a genetic test, recommended or compulsory, in their drug label. Table 1 shows some examples of drugs with pharmacogenomic/genetic marker published in FDA website.

Pharmacogenetic Biomarkers in Drug Labels			
	Therapeutic Area	Biomarker	Label Sections
Aripiprazole	Psychiatry	CYP2D6	Clinical Pharmacology
Azathioprine	Rheumatology	TPMT	Dosage and Administration, Warnings and Precautions, Drug Interactions, Adverse Reactions, Clinical Pharmacology
Carvedilol	Cardiovascular	CYP2D6	Drug Interactions, Clinical Pharmacology
Celecoxib	Analgesics	CYP2C9	Dosage and Administration, Drug Interactions, Use in Specific Populations, Clinical Pharmacology
Cetuximab	Oncology	EGFR	Indications and Usage, Warnings and Precautions, Description, Clinical Pharmacology, Clinical Studies
Cetuximab	Oncology	KRAS	Indications and Usage, Clinical Pharmacology, Clinical Studies
Maraviroc	Antivirals	CCR5	Indications and Usage, Warnings and Precautions, Clinical Pharmacology, Clinical Studies, Patient Counseling Information

Table 1. Examples of pharmacogenetic biomarkers in drug labels in FDA website (www.fda.gov, accession date: (24/06/2011).

On the other hand, Pharmacogenomics is responsible for discovering the relationships between genes and disease by means of the molecular etiology and pathways of each illness. The identification of these new associations opens a window to search for new drugs for novel therapeutic targets.

The aim of both disciplines is to obtain the highest therapeutic effect with the lowest risk, minimizing the adverse effects of the treatments. This should lead to a better control of the disease through personalized medicine.

In order to start explaining the main findings of Pharmacogenetics in the renal transplantation field, we need first to understand some basic concepts. The first one is Polymorphism, which is a monogenic mendelian character that is normally present in the population at least in 1% frequency. It is characterized by the presence of more than one allele in a same gene locus, and consequently, more than one phenotype. The polymorphisms that have a meaning in pharmacogenetics are those who represent different alleles in a gene related to the interaction of a drug with the organism. There are different types of polymorphisms: those where the change from one allele to the variant is only one nucleotide are called SNPs, Single Nucleotide Polymorphisms, and these will be the subject of most part of the research in the field, including our own work. But there are also other kind of polymorphisms as RFLPs, Restriction Fragment Length Polymorphisms, and VNTR, Variable Number Tandem Repeats. SNPs are the most common ones, representing 90% of the whole genetic variability. It is important not to mix this concept up with the term "mutation", although actually they are sometimes mixed. A mutation is usually less frequent than a polymorphism, being present in less than 1% of the population but also a mutation represents a very little part of the whole genetic variability and is mostly associated with the pathology concept: a mutation is most always the cause of a missfunction or disease, while a polymorphism will only show some biological effect under some concrete circumstances. For instance, if we are carriers of a variant in a polymorphic site, related to a better efficacy of a given drug, we will only notice this effect if we take that drug, but if we never take it, we will probably not find any biological effect in our body due to carrying that variant.

Another important concept to consider is the Haplotype. We refer to haplotype when we talk about alleles which tend to be inherited together because they are close to each other on the chromosomes. The haplotypes have been extensively studied by the HapMap project (www.hapmap.org). Currently, the investigations are more and more directed to the study of the effect of several combined SNPs, instead of aisled SNPs, especially those that form an haplotype. The reason for this is that most probably the biological effects that we could find are the result of the sum of the effect of several SNPs in different genes affecting several parts of the pathway of the drug. Moreover, the effect of a single SNP can be, in turn, enhanced, reduced or silenced by compensation by the effect of other SNPs. Related to the term haplotype we can also hear about Linkage Disequilibrium, LD, which is the occurrence of some combinations of alleles or genetic markers in a population more often or less often than would be expected from a random formation of haplotypes from alleles based on their frequencies. This aspect is also important in the study of SNPs, since in many cases, a SNP that apparently seems to have no clear biological meaning may be in disequilibrium with another SNP with a known effect and taking this into account, this property could help us infer the genotype of a patient in several SNPs, by only

studying one or two of them if they are linked, and also it makes sense to study not only the SNPs with a clear direct effect, but those apparently non-functional but linked to the functional ones.

The application of these skills and expertise in the field of transplantation could be one of the great advances in current immunosuppressive therapy. In the transplantation field, there are still many unanswered questions, its success depends on the fragile equilibrium between the risks and benefits of immunosuppression. Therapeutic drug monitoring helps to determine suitable immunosuppressant dose adjustments but usually the work is done by assay-error method so the challenge now is to combine pharmacokinetic with pharmacogenetic information to provide patients with the most suitable treatment. Many papers have been written about genetic variability based on SNPs influencing immunosuppressant blood levels but the results are still contradictory in many cases, probably due to the hidden effects of other SNPs not included in the study, the number of patients included, their ethnicity (SNP frequencies can be very different from one ethnicity to another) and so on.

Just focusing our review of the state of the art in tacrolimus and cyclosporine, two genes seem to be the most relevant on having pharmacogenetic effects on these drugs: the ABCB1 gene, coding for the transporter P-glycoprotein, and the CYP3A5 gene, coding for an extensive drug-metabolizing enzyme of cytochrome P450 family.

Tacrolimus and cyclosporine are calcineurin inhibitors indicated for prophylaxis of renal, liver and heart transplant rejection. Cyclosporine has a broader use as immunosuppressive agent, as apart from preventing transplant rejection (kidney, liver, heart, lung and bone marrow) it is also employed in autoimmune diseases (uveitis endogenous, psoriasis, nephrotic syndrome, rheumatoid arthritis and atopic dermatitis). In the metabolism of both drugs, cytochrome P-450 plays an important role, specifically the CYP3A5 isoform, but also CYP3A4 in the case of cyclosporine. On the other hand, they are both transported out of cells by P-glycoprotein, encoded by the gene ABCB1. Changes in expression or function of these proteins will cause changes in the absorption, metabolism and distribution of both drugs and, therefore, can lead to changes in the response and toxicity of the treatment. The characterization of genetic variants, as for instance SNPs, that cause variations in expression or function can help in establishing effective doses and in minimizing adverse reactions (Astellas Pharma, S.A., 2010; Staatz et al., 2010).

The good correlation between these two drugs blood levels and their tissue concentration, makes them suitable for pharmacokinetic monitoring to prevent graft rejection or toxicity. If we add the inter- and intra-individual variability, a narrow therapeutic range and a clear correlation between high blood levels and appearance of toxicity, then we have two perfect candidates for the need of dose optimization. Other factors affecting tacrolimus and cyclosporine dosage/concentration after renal transplantation are consistent with the features of the patients (renal and hepatic function, age, race). Liver function, albumin, hematocrit, gastrointestinal disturbances and the effects of food are also important factors responsible for the variation of dosage/concentrations (Marqués et al., 2009).

The final goal of pharmacogenetics in transplantation is to find a clear link between genetic variations and pharmacokinetics/pharmacodynamics of the drugs employed, to allow us to find the optimal doses of both initial and maintenance periods, ensuring the success of the

graft and reducing treatment toxicity. Amongst all the SNPs described in the literature included in genes related to these drugs, we need to validate those that really have an impact on actual clinical practice, those that really alter drug levels/efficacy/toxicity and we can only achieve that through research, in order to obtain individualized therapies as effective as possible. Contradictory results in the published studies seem to tarnish the utility of pharmacogenetics, but there are also many encouraging works, where clear correlations are found. Probably a good experimental design, without alteration of the real clinical setting could help to discriminate whether these studies will be useful or not in the therapeutic decisions, but certainly we need multiple approaches correlating genetics with kinetics and with safety/efficacy; good and accurate informatic tools to process such a great quantity of information; and finally, we do not have to forget other important factors involving the patient as concomitant drugs and donor genotype. In this chapter we are presenting a summary of some relevant published works and some of our own group's results in this area.

2. Tacrolimus

2.1 Absorption

Tacrolimus has a mean oral bioavailability ranging from 20 to 25%. After oral administration, peak concentrations (C_{max}) are reached in an interval of 1 to 3 hours. Co-administration with food leads to a decrease in the speed and extent of absorption, being more accentuated in the case of fat rich food. Once in blood, it is highly found bound to erythrocytes and plasma proteins (> 98.8%), with preference for serum albumin and α -1 acid glycoprotein (Astellas Pharma, S.A., 2010).

2.2 Distribution

It is widely distributed throughout the body reaching a volume of distribution around 1,300 liters. It has a low clearance that, as with its half-life, differs depending on the type of transplantation performed and the age of the patients (total clearance in pediatric patients undergoing liver transplantation is twice that of adult patients with the same transplantation type). Generally, it has a long half-life that is affected by variations in clearance rates observed in transplantation patients (Astellas Pharma, S.A., 2010).

2.3 Metabolism

Tacrolimus is extensively metabolized in the liver but also has a minimal metabolism in the intestine. The primary responsible is the cytochrome P450. Several products of metabolism have been identified, but there is only one metabolite with immunosuppressive activity similar to that of tacrolimus, however, it does not contribute to the pharmacological activity, since it has not been detected in systemic circulation (Astellas Pharma, S.A., 2010).

2.4 Elimination

The main route of elimination of tacrolimus is the faeces. Only 2% is excreted in urine. Only 1% of the administered tacrolimus appears unchanged in feces and urine (Astellas Pharma, S.A., 2010).

3. Cyclosporine

3.1 Absorption

The new current formulation of cyclosporine microemulsion compared to conventional forms, has a faster absorption, leading to a reduction of 1 hour in the t_{max} and C_{max} increased by 59%, with an increase of 29% in AUC (Novartis Farmacéutica, S.A., 2010).

3.2 Distribution

Most of the cyclosporine is outside the blood compartment. In the blood it is preferentially distributed in plasma (33 - 47%) and erythrocytes (41-58%). 90% is fixed to plasma proteins, primarily lipoproteins (Novartis Farmacéutica, S.A., 2010).

3.3 Metabolism

Biotransformation of cyclosporin is broad and leads to the formation of about 15 metabolites. There is not a main metabolic pathway and it suffers enterohepatic cycle (Novartis Farmacéutica, S.A., 2010).

3.4 Elimination

It is mainly performed via the bile. Only 6% of the oral dose is excreted in the urine (0.1% as unchanged drug) (Novartis Farmacéutica, S.A., 2010).

4. Genetic polymorphisms

A great number of genes are thought to be involved in different effects of immunosuppressive therapy (www.fda.gov; www.pharmgkb.org). Two genes in particular have demonstrated clear correlations in several studies: ABCB1 (or MDR1), which codes for the transporter P-glycoprotein and CYP3A5, which codes for an extensive drug metabolizing enzyme of the cytochrome P450 family.

4.1 ABCB1 genetic polymorphisms

P-glycoprotein is encoded by the multidrug resistance gene (MDR1), also known as the ABCB1 gene. The protein encoded by this gene is an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. It is responsible for decreased drug accumulation in multidrug-resistant cells and often mediates the development of resistance to anticancer drugs. This protein also functions as a transporter in the blood-brain barrier. ABCB1 is polymorphically expressed, with at least 50 SNPs identified to date (<http://www.ncbi.nlm.nih.gov>).

4.1.1 Influence on tacrolimus pharmacokinetics.

The results of the influence of ABCB1 on the pharmacokinetics of tacrolimus are controversial. Some studies talk about an increase in the ratio C_0 /dose and lower dose requirement in those individuals expressing variant ABCB1 3435 TT regarding the CC variant, related to a possible lower functional activity of P-glycoprotein in the carriers of TT

variant (Staatz et al, 2010). This “3435 C>T” is the common nomenclature for the SNP cataloged as rs1045642 in the SNP database of NCBI website (<http://www.ncbi.nlm.nih.gov>), where C is the ancestral allele (also “wild type”) and most frequent and T the less frequent one and they lead to three different genotypes: CC, CT and TT. In contrast, some other studies have failed to find an association between the ABCB1 3435C>T and changes in tacrolimus blood levels. In a prospective study with 96 renal transplant recipients, the effect of genetic polymorphisms of ABCB1 on tacrolimus whole blood levels was analyzed, concluding that ABCB1 1199G>A, 3435C>T and 2677G>T/A SNPs (rs2229109, 1045642, 2032582, respectively), appeared to reduce the activity of P-glycoprotein towards tacrolimus, increasing tacrolimus peripheral blood mononuclear cell concentrations. Nevertheless, the impact of ABCB1 genetic polymorphisms on tacrolimus blood concentrations was negligible (Capron et al., 2010). In another study on Chinese renal transplant recipients, MDR1 3435C>T polymorphism was not an important factor in tacrolimus pharmacokinetics (Rong et al., 2010). In a retrospective study of 81 renal transplant recipients the effect on tacrolimus dosages and concentration/dose ratio of four frequent MDR1 SNP possibly associated with P-gp function (T-129C in exon 1b, 1236C>T in exon 12, 2677G>T,A in exon 21, and 3435C>T in exon 26; corresponding to rs3213619, 1128503, 2032582 and 1045642, respectively). In the general caucasian population, the SNP in exons 12, 21, and 26 exhibited incomplete linkage disequilibrium, which means that the different variants of each SNP tend to be displayed together, but not in the 100% of the

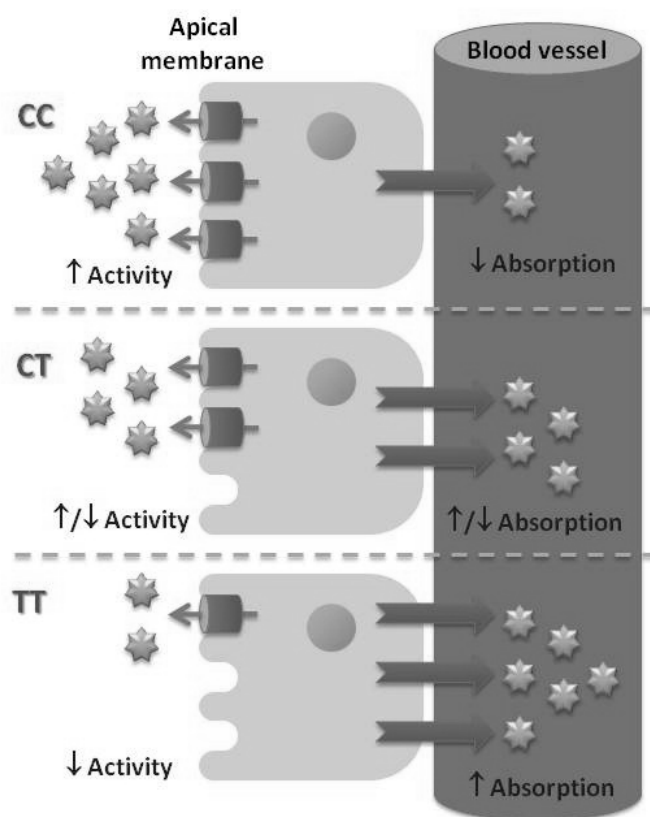


Fig. 1. Influence of the functional activity of glycoprotein-P (transporter in apical membrane) in the transport of tacrolimus (stars) in the intestine epithelium. The diagram shows the different degree of drug absorption due to variations in ABCB1/MDR1 polymorphic site rs1045642. Individuals with TT variant have a decreased transporter activity and hence greater absorption efficiency. CC variant causes more expulsion out of the cell, which decreases absorption.

individuals. One month after tacrolimus introduction, exon 21 SNP correlated significantly with the daily tacrolimus dose ($P < \text{or} = 0.05$) and the concentration/dose ratio ($P < \text{or} = 0.02$). Tacrolimus dose requirements were 40% higher in homozygous TT than in wild-type patients GG for this SNP. The concentration/dose ratio was 36% lower in the wild-type GG patients, suggesting that, for a given dose, their tacrolimus blood concentration is lower. Haplotype analysis substantiated these results and suggested that exons 26 and 21 SNPs may be associated with tacrolimus dose requirements (Anglicheau et al., 2003).

4.1.2 Influence on cyclosporine pharmacokinetics

Nowadays the data about ABCB1 influence on cyclosporine pharmacokinetics are not conclusive, either. Many studies have tried to find correlations with ABCB1 SNPs 3434C>T, 2677G>T/A and 1236C>T without significant findings. A recent meta-analysis that included 1036 renal transplantation recipients, concluded that there were no significant differences in the influence of ABCB1 3435C>T on cyclosporine pharmacokinetics (AUC₄/Dose, CL/F, C_{max}/Dose or C₀/Dose). However, it was indicated in this meta-analysis that CC carriers had lower cyclosporine exposure presented as AUC 0–12 than those with at least one T allele (CT or TT). In a recent study that included 225 renal transplant recipients treated with cyclosporine, ABCB1 2677G>T SNP correlated significantly with dose-adjusted levels in patients, at 1, 3 and 6 months after renal transplantation. Recipients with the wild-type genotype of this SNP were associated with significantly lower dose-adjusted values and consequently required higher cyclosporine daily dose to attain the therapeutic level. ABCB1 1236C>T also had a minor influence on dose-adjusted C₂/T₀ levels (Singh et al, 2010).

4.2 CYP450 genetic polymorphisms

The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. These proteins are localized in the endoplasmic reticulum and their expression is induced by glucocorticoids and some pharmacological agents (<http://www.ncbi.nlm.nih.gov>).

CYP3A5 is found in the liver, small intestine and kidney. One of the most relevant SNPs in this gene is rs776746, also known as 6986 A>G, being G the most frequent allele. Allele *3 is the wild type genotype, GG, while *1 is the variant homozygous AA and heterozygote A/G is known as *1/*3. There are clear ethnic differences in the prevalence of the CYP3A5*3 genotype. At the molecular level, the SNP is located in intron 3, and the change of base produces a splicing defect. The result is a nonfunctional protein (allele *3). The patients that show the allelic variant *3 in homozygosis G/G, also called non-expressors, are slow metabolizers of the immunosuppressant. In contrast, heterozygote A/G alleles *1/*3 are intermediate metabolizers, whereas those carriers of allele *1 in homozygosis A/A are normal metabolizers (Glowacki et al, 2011; Macphee et al, 2005; www.pharmgkb.org)

4.2.1 Influence on tacrolimus pharmacokinetics

CYP3A5 may play a more important role than CYP3A4 in the metabolism of tacrolimus in individuals who are CYP3A5 expressors, so we will focus on this enzyme. The intrinsic clearance of tacrolimus is approximately 2-fold higher for CYP3A5 than for CYP3A4 (Hesselink et al., 2008). This plasmatic clearance is higher in those individuals with genotype CYP3A5*1/*3 regarding those CYP3A5*3/*3 (Haufrond et al., 2006). In fact, CYP3A5*1 is

responsible from about 60% of tacrolimus hepatic metabolism (Dai et al., 2006; Thervet et al., 2010), so it is of great interest studying it in order to establish optimal doses that reach quickly the efficient blood concentrations, avoiding toxicity but also assuring the necessary concentrations to avoid rejection. The studies performed so far indicate the need to administrate higher tacrolimus doses in patients CYP3A5*1/*1. In fact, in some of those studies there is even a recommendation of an initial double dose for those patients (Haufroid et al., 2006). In a study with forty kidney recipients, CYP3A5*1 variant was associated with significant lower tacrolimus dose adjusted concentration at 3, 6, 12 and 36 months after transplantation, concluding that CYP3A5*1 carriers need higher tacrolimus dose than CYP3A5*3 homozygotes to achieve the target blood concentration (Katsakiori et al., 2010). Similarly, in a study with 28 chinese renal transplant recipients, the patients with *1/*3 showed significantly lower tacrolimus blood levels than those with the *3/*3 in the first and second week after transplantation (Zhang et al., 2010). In another similar study, on Chinese renal transplant recipients, individuals who were CYP3A5*1 carriers required a higher dose of tacrolimus than CYP3A5*3/*3, indicating a significantly lower dose-adjusted AUC(0-12) of tacrolimus (Rong et al., 2010). The last reviewed work, with 200 patients objectifies that patients who were CYP3A5*3/*3 received significantly higher tacrolimus dose at 1 week, 6 months, and 1 year (Tavira et al., 2011). Some studies found that the weighted mean apparent oral clearance was 48% lower in CYP3A5 nonexpressors than CYP3A5 expressors (range, 26%-65%) (Barry & Levine, 2010). Recently, a study with 280 transplant recipients concludes that patients receiving a pharmacogenetic adaption of the daily dose of tacrolimus were associated with improved achievement of the target C_0 (Thervet et al., 2010).

4.2.2 Influence on cyclosporine pharmacokinetics

Unlike tacrolimus, CYP3A4 may play a more dominant role than CYP3A5 in the metabolism of cyclosporine. The intrinsic clearance of cyclosporine, calculated from total metabolite formation, is approximately 2.3-fold higher for CYP3A4 than for CYP3A5 (Dai et al., 2006). Still, the results of studies conducted so far do not indicate a clear relationship (Anglicheau et al., 2007) with 3A4 isoform. However, many papers continue studying the effect of both on the metabolism of cyclosporine. A recent meta-analysis with twelve studies includes the effects of CYP3A5 during cyclosporine dose adjustment. This meta-analysis showed that CYP3A5*3/*3 polymorphism is associated with cyclosporine dose-adjusted concentration (dose-adjusted trough and dose-adjusted peak concentrations) in renal transplant recipients and patients carrying the CYP3A5*3/*3 genotype will require a lower dose of cyclosporine to reach targets levels compared with the CYP3A5*1/*1 or 1*/*3 carriers (Zhu et al., 2011).

5. Our results

5.1 Objective

The aim of our studies was to evaluate the effect of the most relevant SNPs in ABCB1 and CYP3A5 genes, in renal transplant recipients and their donors, regarding blood concentrations of tacrolimus in the first two weeks post-transplantation.

5.2 Materials and methods

One blood sample, collected in anticoagulation tubes at routine extraction, was obtained in each of 97 renal transplant recipients and their donors (caucasians). The DNA was extracted

from 200 μ L of blood using a commercially available kit based on centrifugation in microcolumns (UltraClean BloodSpin DNA Isolation Kit; MoBioLaboratories, Inc, Carlsbad, California). After quantification using a spectrophotometer (NanoDrop Technologies Inc, Wilmington, Delaware) to determine the concentration and purity, DNA was stored at -20°C until use. A genetic analysis platform (MassARRAY; Sequenom, Inc, San Diego, California) was used to obtain the genotypes of each sample in the SNPs rs1045642 (3435C>T), rs2032582 (2677G>T/A), and rs1128503 (1236C>T) of the ABCB1 gene, and in the SNPs rs776746 (6986A>G, CYP3A5*3) and rs10264272 (267871G>A, CYP3A5*6) of the CYP3A5 gene. All the 97 patients received tacrolimus as the primary immunosuppression drug, at an initial dose of 0.2 to 0.3 mg/kg/24 h. Blood concentration of tacrolimus was measured routinely using a clinical chemistry system (Dimension; Siemens Healthcare, Deerfield, Illinois) to determine the trough level (C_0 , in nanograms per milliliter). Drug blood concentrations from the first and second weeks were determined, and all of the measured levels in the patients during that period were considered. The resulting values were plotted in the form of median and quartile range, 1 for each week in each group of recipients or recipient with their donors, according to genotypes. Normality tests of Kolmogorov-Smirnov and Shapiro-Wilk were performed and then differences between groups were evaluated using the Mann-Whitney test (nonparametric test that compares two groups)

5.3 Results

The SNP genotyping of the 167 samples showed similar frequencies to those expected for Caucasian population in public databases (SNP Database in NCBI site). Tables 2 to 4 show the relationship between the frequencies observed in recipients in our study regarding the expected ones according to the data from public data bases of the ABCB1 and CYP3A5 genes, in the most relevant genotyping assays in caucasian population. Tables 5 to 7 show the same information but regarding the donors of the transplanted kidneys. The data related to donors is a little bit outside the expected range, while there is a better fit in recipient's data. We associate these findings to the lower number of donors, thus we can appreciate that in recipients, having a larger number of samples, the frequencies fit better.

GENE	POLYMORFISMS	EXPECTED FREQUENCY (%)			FOUND FREQUENCY (%)		
		CC	CT	TT	CC	CT	TT
ABCB1	rs 1045642	12.5-15.5	50-60.3	37.5-24.1	22.68	52.28	24.74
	rs 1128503	32	41	27	34.02	46.39	24.05

Table 2. Recipients' genotypes frequency regarding the expected frequencies from public data bases.

GENE	POLYMORFISMS	EXPECTED FREQUENCY (%)					FOUND FREQUENCY (%)				
		AA	AG	GG	GT	TT	AA	AG	GG	GT	TT
ABCB1	rs 2032582	0	0	32.3-32.2	48.4-55.9	19.3-11.9	1.03	1.03	32.99	50.52	14.43

Table 3. Recipients' genotypes frequency regarding the expected frequencies from public data bases.

GENE	POLYMORFISMS	EXPECTED FREQUENCY (%)		FOUND FREQUENCY (%)	
		GG	GA	GG	GA
CYP3A5	rs 776746	88.3-95.5	11.7-4.5	81.44	18.55
	rs 1026427	100	0	97.94	2.06

Table 4. Recipients' genotypes frequency regarding the expected frequencies from public data bases.

GENE	POLYMORFISM S	EXPECTED FREQUENCY (%)			FOUND FREQUENCY (%)		
		CC	CT	TT	CC	CT	TT
ABCB1	rs 1045642	12.5-15.5	50-60.3	37.5-24.1	30.07	49.18	14.75
	rs 1128503	32	41	27	39.06	50	9.38

Table 5. Donors' genotypes frequency regarding the expected frequencies from public data bases.

GENE	POLYMORFISMS	EXPECTED FREQUENCY (%)					FOUND FREQUENCY (%)				
		AA	AG	GG	GT	TT	AA	AG	GG	GT	TT
ABCB1	rs 2032582	0	0	32.3-32.2	48.4-55.9	19.3-11.9	0	0	42.37	54.24	3.39

Table 6. Donors' genotypes frequency regarding the expected frequencies from public data bases.

GENE	POLYMORFISMS	EXPECTED FREQUENCY (%)		FOUND FREQUENCY (%)	
		GG	GA	GG	GA
CYP3A5	rs 776746	88.6-95.5	11.4-4.5	79.10	19.4
	rs 1026427	99	1	98.5	1.43

Table 7. Donors' genotypes frequency regarding the expected frequencies from public data bases.

5.3.1 ABCB1

According to the literature, rs1045642 seems to be the most relevant SNP in ABCB1 gene regarding its correlation with tacrolimus or cyclosporine blood levels.

Figure 2 shows tacrolimus levels as trough concentration, corrected by administered dose and weight of the patient ($C_0/D_c = C_0 / (\text{Dose} / \text{weight})$) during the first two weeks after renal transplantation, grouped in a way that we represent the data of those recipients whose donor has the same genotype as them (eg. CC/CC, means recipients CC whose donors were also CC). The data are represented as median inside the whole data range, remarking the interquartile range which includes the 50% of the data. The statistical test applied was Mann-Whitney two-tailed test. Statistically significant differences ($p < 0,05$) were found between CC/CC vs TT/TT and also between CT/CT vs TT/TT in the second week after transplantation. With this kind of analysis we evaluate the global effect of the variant, C or T, without taking into account if the effect is greater in recipients or in donors.

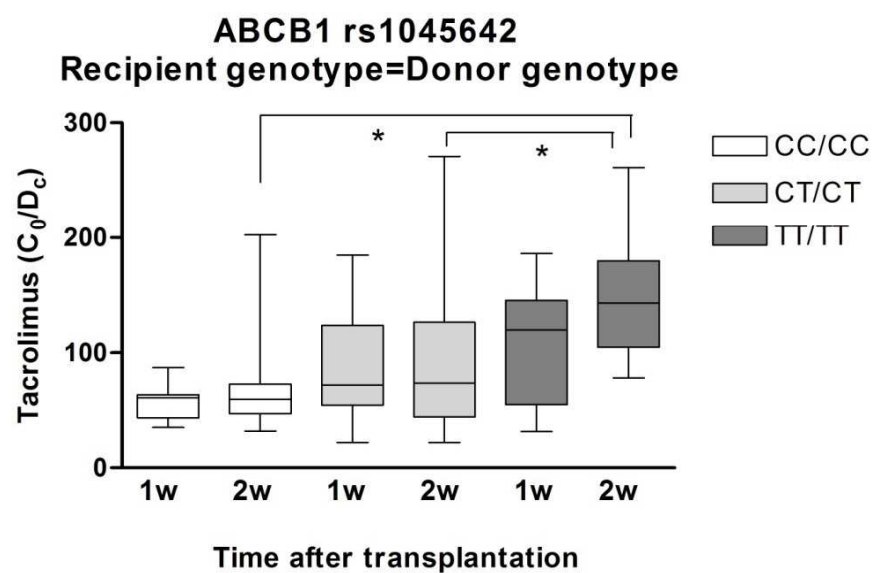


Fig. 2. Tacrolimus corrected trough concentration in renal recipients regarding single nucleotide polymorphism (SNP) rs1045642 genotype recipient/ donor. Corrected trough concentration ($C_0/(\text{dose}/\text{weight})$) (C_0 : ng/mL; dose: mg/Kg/day; weight: Kg) regarding the patient and donor genotype, grouped so that both genotypes match, in SNP rs1045642 of ABCB1 gene, in the first (1w) and second week (2w) after transplantation. The horizontal line inside each bar is the median of the data, the bar is the interquartile range, including 50% of the data, and the lines up and down the bars cover the whole data range for each set. A significant difference ($*= p < 0,05$) was achieved in the second week between CC/CC and TT/TT, and also between CT/CT and TT/TT employing Mann-Whitney two-tailed test.

Figures 3, 4 and 5 show the same kind of analysis as in Figure 2 but with patients grouped by a single recipient variant comparing the three possible donors' genotype.

We could not find any statistically significant differences in Fig.3, applying Mann-Whitney non-parametric test, most probably because we only had one value for CC/TT in the first week, and two for the second. However, those three values show the expected trend of higher tacrolimus levels than the rest of the groups.

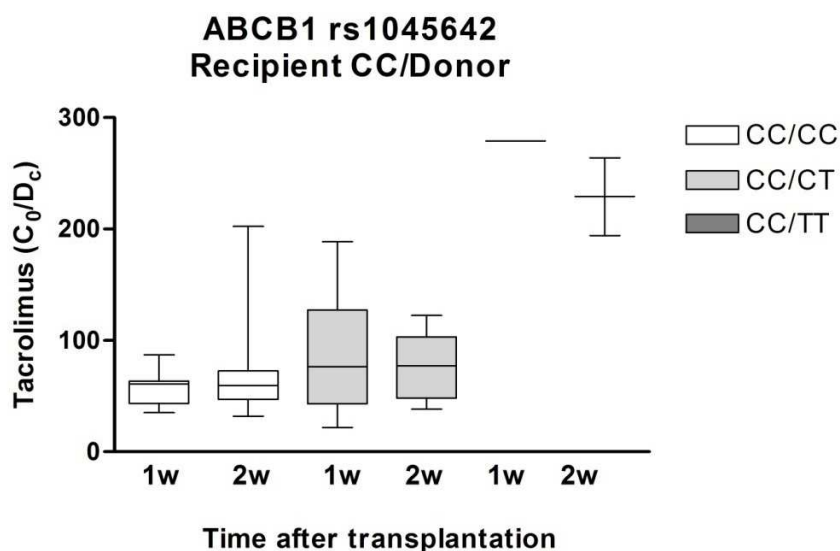


Fig. 3. Tacrolimus corrected trough concentration in renal recipients regarding single nucleotide polymorphism (SNP) rs1045642 in CC recipients. Corrected trough concentration ($C_0/(\text{dose}/\text{weight})$) (C_0 : ng/mL; dose: mg/Kg/day; weight: Kg) regarding the patient and donor genotype, showing the data from recipients CC divided in three different groups according to their donor genotype (recipient/donor), in the first (1w) and second week (2w) after transplantation. The horizontal line inside each bar is the median of the data, the bar is the interquartile range, including 50% of the data, and the lines up and down the bars cover the whole data range for each set. No significant differences were found.

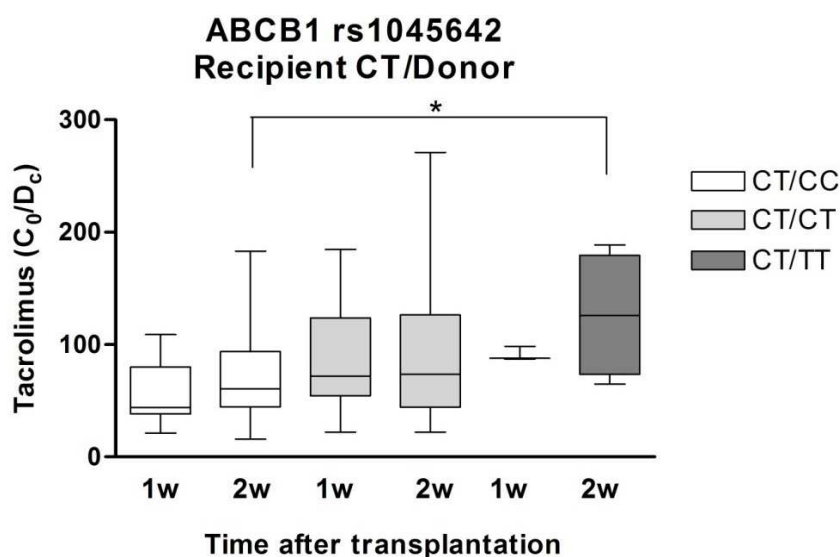


Fig. 4. Tacrolimus corrected trough concentration in renal recipients regarding single nucleotide polymorphism (SNP) rs1045642 in CT recipients. Corrected trough concentration ($C_0/(\text{dose}/\text{weight})$) (C_0 : ng/mL; dose: mg/Kg/day; weight: Kg) regarding the patient and donor genotype, showing the data from recipients CT divided in three different groups according to their donor genotype (recipient/donor), in the first (1w) and second week (2w) after transplantation. The horizontal line inside each bar is the median of the data, the bar is the interquartile range, including 50% of the data, and the lines up and down the bars cover the whole data range for each set. Significant differences were found between CT/CC and CT/TT at week two ($p < 0,05$) employing Mann-Whitney two-tailed test.

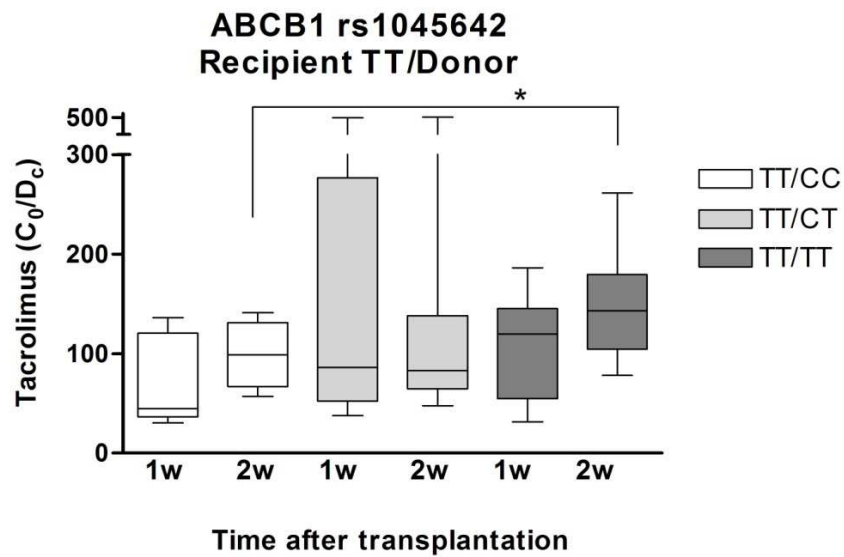


Fig. 5. Tacrolimus corrected trough concentration in renal recipients regarding single nucleotide polymorphism (SNP) rs1045642 in TT recipients. Corrected trough concentration ($C_0/(dose/weight)$) (C_0 : ng/mL; dose: mg/Kg/day; weight: Kg) regarding the patient and donor genotype, showing the data from recipients TT divided in three different groups according to their donor genotype (recipient/donor), in the first (1w) and second week (2w) after transplantation. The horizontal line inside each bar is the median of the data, the bar is the interquartile range, including 50% of the data, and the lines up and down the bars cover the whole data range for each set. Significant differences were found between TT/CC and TT/TT at week two ($p < 0,05$) employing Mann-Whitney one-tailed test.

There is a clear trend of CC genotype in SNP rs1045642 to normalize tacrolimus levels, while there is always an increase related with T allele, especially in TT genotype, where most of the times, statistically significant differences are reached at week two post-transplantation. With these data, we can calculate an increase in the value of the median up to 286.53% when comparing the difference between the median in group CC/TT and the median in group CC/CC (fig. 2, second week) Table 8 shows the differences in percentage of the medians from figures 2, 3, 4 and 5, and also the quantity of recipients and tacrolimus level values included in each of the figures.

But we have not only studied rs1045642, also two other relevant SNPs in ABCB1 that have been described to be in linkage disequilibrium with the first: rs1128503 and rs2032582. With the genotype data for these three SNPs in our renal transplantation recipients, we constructed haplotype groups, being "Normal" those recipients carrying CC in rs1045642, CC in rs1128503 and GG in rs2032582, and "Variant" those carrying the rest of the possible combinations, containing at least one T allele in any of the three SNPs. The results correlating these genotypes with tacrolimus corrected trough level are shown in figures 6 and 7. In figure 6 the data are represented according to the donors' haplotype, with $n=23$ values for "normal" group in the first week and 29 in the second, and for "variant", 77 in the first week and 93 in the second. Figure 7 shows the data arranged according to the haplotype of the recipients, with 21, 19, 86 and 110 values, respectively. In both figures we find significant differences of $p < 0,05$ between the two groups with Mann-Whitney two-tailed test.

GENOTYPE 1 (R/D)*	N° PATIENTS	N° SAMPLES	GENOTYPE 2 (R/D)*	N° PATIENTS	N° SAMPLES	Δ%
TT/TT	4	7	CC/CC	6	12	141.4
			CT/CT	15	33	94.6
			TT/CC	5	8	44.53
			TT/CT	9	20	71.88
CC/TT	1	2	CC/CC	6	12	286.53
			CC/CT	8	14	196.52
CT/TT	2	4	CT/CC	14	29	107.52
			CT/CT	15	33	71.29

Table 8. Percent increase ($\Delta\%$) of the median in the second week after transplantation, comparing different groups according to their genotype recipient/donor, as shown in figures 2-5. Columns 2 and 5 show the number of recipients in each group, and columns 3 and 6 show the number of tacrolimus levels included. *Recipient/Donor.

ABCB1 Donor's Haplotype

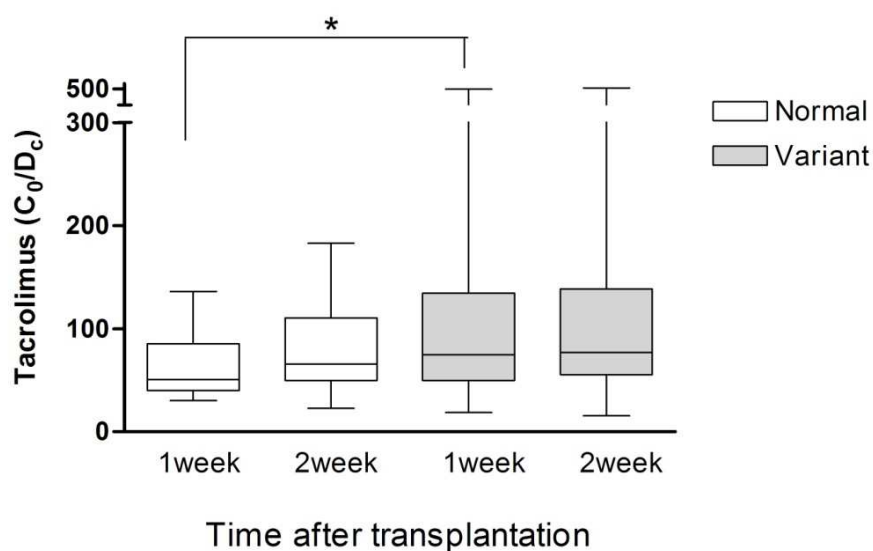


Fig. 6. Tacrolimus corrected trough concentration in renal recipients regarding SNPs rs1045642, rs1128503 and rs2032582 haplotype in their donors. Corrected trough concentration ($C_0 / (\text{dose} / \text{weight})$) (C_0 : ng/mL; dose: mg/Kg/day; weight: Kg) regarding the patient's donor genotype, showing the data from recipients divided in two groups according to their donor's genotype being Normal those whose donors are CC/CC/GG in the three SNPs respectively, and Variant those with any of the other possible combinations. The results displayed correspond to the first (1w) and second week (2w) after transplantation. The horizontal line inside each bar is the median of the data, the bar is the interquartile range, including 50% of the data, and the lines up and down the bars cover the whole data range for each set. Significant differences were found between the two groups at the first week ($p < 0,05$) employing Mann-Whitney two-tailed test.

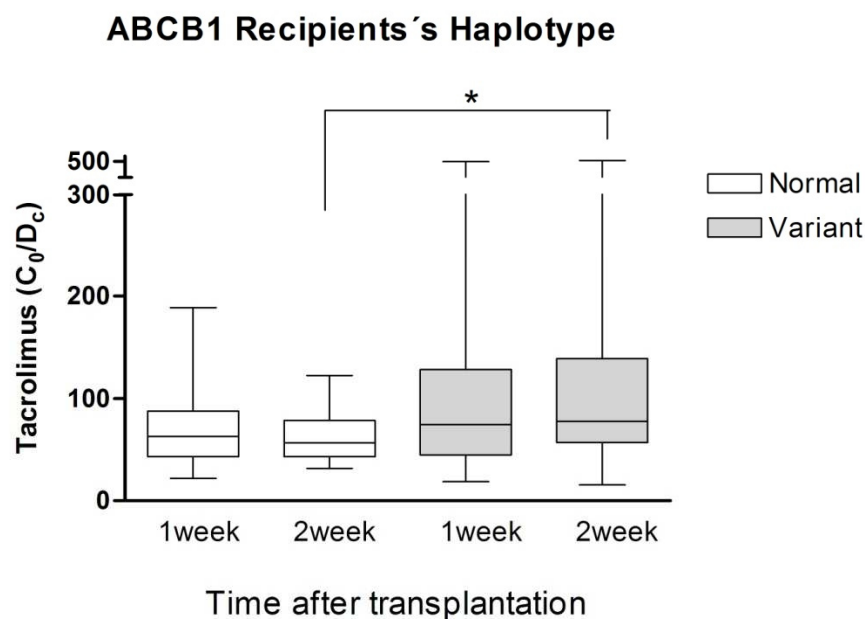


Fig. 7. Tacrolimus corrected trough concentration in renal recipients regarding SNPs rs1045642, rs1128503 and rs2032582 haplotype in the patients. Corrected trough concentration ($C_0 / (\text{dose} / \text{weight})$) (C_0 : ng/mL; dose: mg/Kg/day; weight: Kg) regarding the recipients' genotype, showing the data from recipients divided in two groups according to their haplotype being Normal those CC/CC/GG in the three SNPs respectively, and Variant those with any of the other possible combinations. The results displayed correspond to the first (1w) and second week (2w) after transplantation. The horizontal line inside each bar is the median of the data, the bar is the interquartile range, including 50% of the data, and the lines up and down the bars cover the whole data range for each set. Significant differences were found between the two groups at the second week ($p < 0,05$) employing Mann-Whitney two-tailed test.

5.3.2 CYP3A5

The corresponding data for the analysis of SNPs rs776746 and rs10264272 of CYP3A5 showed the expected behavior, already described in the literature. In figure 8, we find a significant increase in tacrolimus concentration in patients GG, the non-expressors, regarding GA. We failed to have AA patients, which we would expect to have even lower concentrations than GA. Regarding SNP rs102642272, we only had one GA patient, so we could not perform any statistical analysis, however the only three values that we have from that recipient are consistent with the expected results of higher tacrolimus concentrations.

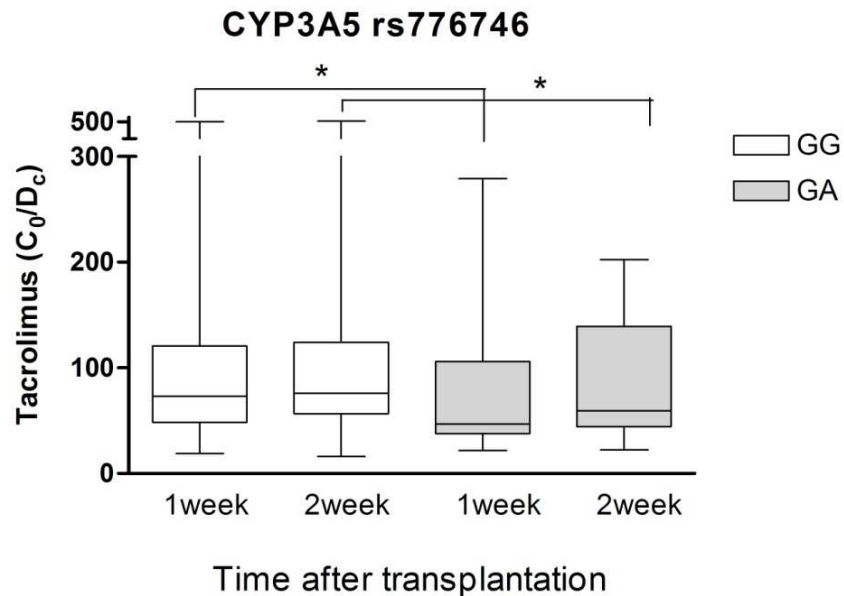


Fig. 8. Tacrolimus corrected trough concentration in renal recipients regarding SNP rs776746 genotype. Tacrolimus corrected trough concentration (C_0/D_c (dose/weight)) (C_0 ng/mL; dose mg/Kg/24 hours; weight Kg) is shown regarding recipient's genotype GG or GA, in the first (1w) and second (2w) week after transplantation. $P < 0,05$ statistically significant difference was found between groups at both weeks by Mann-Whitney one-tailed test.

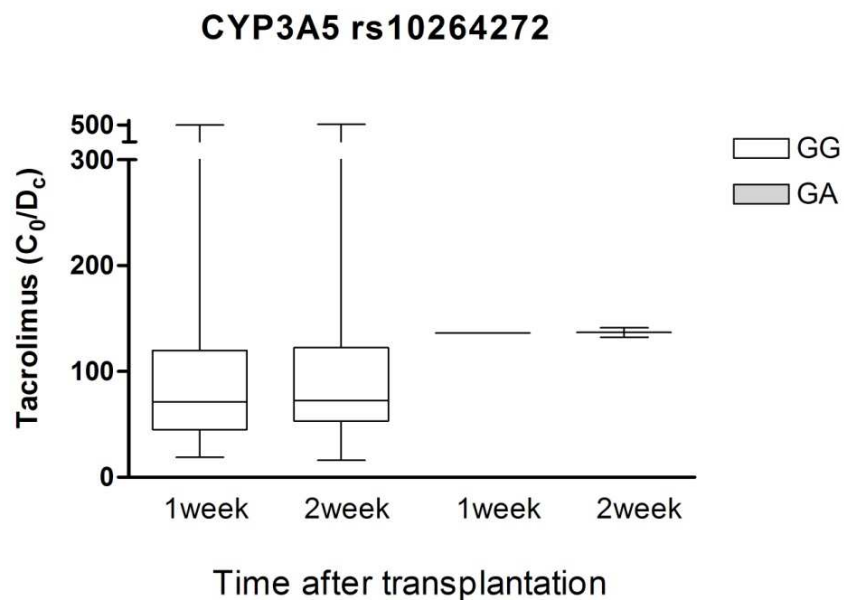


Fig 9. Tacrolimus corrected trough concentration in renal recipients regarding SNP rs10264272 genotype. Tacrolimus corrected trough concentration (C_0/D_c (dose/weight)) (C_0 ng/mL; dose mg/Kg/24 hours; weight Kg) is shown regarding recipient's genotype GG or GA, in the first (1w) and second (2w) week after transplantation. No statistical difference was found between groups.

6. Conclusions

The importance of obtaining the best results in terms of efficacy and safety, but also in terms of economic saving is out of discussion. With the personalized medicine we search for formulas that help us decide which is the perfect treatment in drug and dose for each patient. This is actually possible nowadays with the help of tools as pharmacogenetics, but there is still a lot of work to do before this tool would really be useful in all the therapeutic areas in the daily work with the patient. There are a lot of published works about many genes and SNPs, but when we look deeply for the usefulness, not all of them pass the test. In this work we have confirmed with real patients' data, the expected effect of five different SNPs in ABCB1 and CYP3A5 genes. We have also demonstrated that there is an additional effect of the donor's genotype translated into the received organ, which is also playing a role in the transport (ABCB1) of tacrolimus (Herrero et al., 2010).

But there are still many other genes related to tacrolimus, and also to other immunosuppressants as cyclosporine and micophenolic acid, which have to be explored and validated in the real clinical setting.

Interdisciplinary groups are also necessary for a complete approach to the scenario, only summing the work of different professionals could we achieve the success. The results in other kinds of transplantation must also be taken in consideration (Jordán et al, 2011).

The effects of many other factors need also to be considered, as they may overlap or disguise a genotype effect.

With the data in our hands, we have found a clear effect of increased tacrolimus trough concentration associated with TT genotype in SNPs rs1045642, rs1128503 and rs2032582 in ABCB1 gene. And regarding CYP3A5, we have also found an increase of the mentioned levels in the non-expressor genotype GG. These data, in agreement with more published works by other groups (Haufroid et al. 2006, Thervet et al., 2010), makes us consider conducting an analysis to any patient before initiating an immunosuppressive therapy, before the transplantation is performed in order to know in advance the optimal dose based on the polymorphisms found.

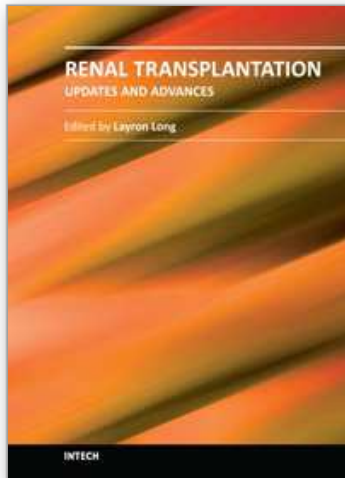
Further studies with a higher number of patients, also in long term responses, relating several SNPs at the same time, building haplotypes and taking also into consideration the correlation with adverse effects are required to establish this "new" and promising Pharmacogenetic Tool.

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This book presents a nice international compilation of scholarly papers and chapters which address the latest advances in renal transplant surgery. These works cover a variety of topics; the last advance and success of renal transplant science: biochemistry, immunology, molecular genetics, pharmacology - pharmacogenetics, pediatric transplant and a few rare uropathies that warrant organ replacement.

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