SNPs PROFILING OF RENAL ALLOGRAFT RECIPIENTS WITH ACUTE REJECTION

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Background

Acute renal allograft rejection is define as a sudden decline in the function of the transplanted kidney, that gives rise to the creatinine levels. Whenever acute rejection is confirmed, the possibility of inadequate immunosuppression, whether due to inadequate dosing or noncompliance, must be addressed. Transplant outcomes exhibit substantial inter-individual variability among patients receiving the same immunosuppressive medications. Recent studies have shown that single-nucleotide polymorphisms (SNPs) in genes involved in immune responses and in the pharmacokinetics/pharmacodynamics of immunosuppressive drugs are associated with allograft rejection in kidney transplantation recipients. Pharmacokinetics of calcineurin inhibitors (like Tacrolimus) are influenced by the multi-drug resistance 1 transmembrane pump (ABCB1/MDR1). The immunosuppressive agent mycophenolic acid (MPA) is metabolized by uridine diphosphate glucuronosyltransferase 1A9 (UGT1A9). Several polymorphisms in this gene have been reported to enhance the glucuronidation of MPA that in turn, results in a lower MPA exposure. Inhibition of IMPDH2 by MPA constitutes part of an immunosuppressive therapy in kidney allograft recipients. The polymorphic variants of this gene have been associated with increased IMPDH2 activity and reduced ability of MPA to exert antiproliferative effects on lymphocytes. Cytokines, like tumor necrosis factor-alpha (TNF-alpha) and interleukin-10 (IL-10), play a key role in the pathogenesis of renal reperfusion injury and organ failure, and are produced in the graft during rejection. The aim of the study was to determine the SNPs profiles associated with the acute rejection event in the kidney transplanted patients.

Methods

We performed an observational, retrospective, non-matched Case-Control Study. Genomic DNA was isolated from 200 µl of whole blood samples collected in 3 experimental groups; Case: kidney transplant patients with AR event(s), Control I: kidney transplant patients without AR event(s), Control II: healthy blood donors. 19 SNPs in 5 genes involved in immune responses and in the pharmacokinetics/pharmacodynamics of immunosuppressive drugs associated with acute renal allograft rejection were studied: 1-1 SNPs in 2 cytokines, TNF-alpha and IL-10; 3 SNPs related to the ABCB1/MDR-1; 10 SNPs connected to IMPDH2; 4 SNPs in UGT1A9 enzyme. All SNPs were analyzed by Sanger sequencing method. Statistical analysis of allele and genotype frequencies and trend test were performed between Case vs Control I; Transplant (Case+Control I) vs Control II; Case vs Control II groups. $p<0.05$ was considered as statistically significant.

Results

Characteristics of the population

A total number of 220 individuals were included in the analysis: 70 healthy blood donors, 109 transplants patients without AR and 41 transplants patients with AR. The characteristics of our groups are summarized in Table 1.

Table 1

Characteristics	Case (41)	Control I (109)	Control II (70)	p-value
Gender: Male Female	 36 (88%) 5 (12%)	 67 (62%) 42 (38%)	 54 (77%) 16 (23%)	0.003
Median age (p25th-p75th)	50 (42-62)	55 (48-62)	49 (41-54)	0.012

Heterozygosity and Hardy-Weinberg equilibrium (HWE) were calculated separately for each group. All studied polymorphisms are in Hardy-Weinberg equilibrium (HWE), except the rs1045642 (p=0,01) of ABCB1/MDR1 in the Case group as shown in the Table 2.

Table 2

GENE	SNP	CHROMOSOME	HET observed	HWE p value
IL-10	rs1800872	Chr1	0.32	p= 0.75
TNFα	rs1800629	Chr6	0.17	p= 0.42
ABCB1/MDR1	rs2032582	Chr7	0.54	p= 0.30
ABCB1/MDR1	rs1128503	Chr7	0.49	p= 0.25
ABCB1/MDR1	**rs1045642**	**Chr7**	**0.61**	**p= 0.01**
UGT1A9	rs2741045	Chr2	0.34	p= 0.62
UGT1A9	rs2741046	Chr2	0.34	p= 0.62
UGT1A9	rs6714486	Chr2	0.03	p= 0.93
UGT1A9	rs17868320	Chr2	0.05	p= 0.87
IMPDH2	rs11706052	Chr3	0.22	p= 0.65

Genotyping

The observed allele frequencies, calculated for each SNPs in the 3 groups, are in line with ones reported from European population; there is a similar trend in the observed genotypes frequencies in all 3 groups: the more frequent genotype in a group is the same in the other groups.

Genetic association

We analyzed all SNPs per-allele, per-genotype and we performed linear trend test between all three groups. There are no statistically significant differences between Case and Control I groups (Table 3), and not even between all transplant patients (Case + Control I) and Control II (Table 4).

Table 3. Genetic association between Case and Control I groups.

GENE	SNP	CHROMOSOME	p-value per Genotype analysis	p-value per Allele analysis	p-value Linear Trend analysis
IL-10	rs1800872	Chr1	p= 0.41	p= 0.23	p= 0.25
TNFα	rs1800629	Chr6	p= 0.62	p= 0.80	p= 0.80
ABCB1/MDR1	rs2032582	Chr7	P= 0.35	p= 0.36	p= 0.35
ABCB1/MDR1	rs1128503	Chr7	p= 0.36	p= 0.21	p= 0.19
ABCB1/MDR1	rs1045642	Chr7	p= 0.05	p= 0.09	p= 0.07
UGT1A9	rs2741045	Chr2	p= 0.23	p= 0.10	p= 0.12
UGT1A9	rs2741046	Chr2	p= 0.23	p= 0.10	p= 0.12
UGT1A9	rs6714486	Chr2	p= 0.59	p= 0.59	p= 0.59
UGT1A9	rs17868320	Chr2	p= 0.45	p= 0.45	p= 0.45
IMPDH2	rs11706052	Chr3	p= 024	p= 0.62	p= 0.60

Table 4. Genetic association between Case+Control I and Control II groups

GENE	SNP	CHROMOSOME	p-value per Genotype analysis	p-value per Allele analysis	p-value Linear Trend analysis
IL-10	rs1800872	Chr1	p= 0.13	p= 0.06	p= 0.06
TNFα	rs1800629	Chr6	p= 0.71	p= 0.45	p= 0.45
ABCB1/MDR1	rs2032582	Chr7	P= 0.12	p= 0.13	p= 0.14
ABCB1/MDR1	rs1128503	Chr7	p= 0.13	p= 0.19	p= 0.18
ABCB1/MDR1	rs1045642	Chr7	p= 0.07	p= 0.07	p= 0.06
UGT1A9	rs2741045	Chr2	p= 0.66	p= 0.40	p= 0.42
UGT1A9	rs2741046	Chr2	p= 0.80	p= 0.96	p= 0.96
UGT1A9	rs6714486	Chr2	p= 0.18	p= 0.19	p= 0.18
UGT1A9	rs17868320	Chr2	p= 0.23	p= 0.23	p= 0.22
IMPDH2	rs11706052	Chr3	p= 0.29	p= 0.13	p= 0.12

Since patients currently without acute rejection events may have acute rejection episodes in the future we also compared Case to Control II group. There are statistically significant differences between Case and Control II groups for 2 SNPs in the ABCB1/MDR1 gene (bold letter in Table 5). Studies in various pharmacological treatments show that carries of C/C an C/T genotypes are considered to be high pumps and have a higher pump activity than those with low-pump (related to T).

Table 5. Genetic association between Case and Control II groups

GENE	SNP	CHROMOSOME	p-value per Genotype analysis	p-value per Allele analysis	p-value Linear Trend analysis
IL-10	rs1800872	Chr1	p= 0.10	p= 0.03	p= 0.03
TNFα	rs1800629	Chr6	p= 0.55	p= 0.48	p= 0.48
ABCB1/MDR1	**rs2032582**	**Chr7**	**P= 0.02**	**p= 0.03**	**p= 0.04**
ABCB1/MDR1	rs1128503	Chr7	p= 0.09	p= 0.07	p= 0.07
ABCB1/MDR1	**rs1045642**	**Chr7**	**p= 0.01**	**p= 0.01**	**p= 0.01**
UGT1A9	rs2741045	Chr2	p= 0.21	p= 0.08	p= 0.08
UGT1A9	rs2741046	Chr2	p= 0.45	p= 0.23	p= 0.22
UGT1A9	rs6714486	Chr2	p= 0.23	p= 0.24	p= 0.23
UGT1A9	rs17868320	Chr2	p= 0.73	p= 0.73	p= 0.73
IMPDH2	rs11706052	Chr3	p= 0.23	p= 0.13	p= 0.13

Conclusions

Analysis of allele and genotype frequencies and gene-disease association tests showed that patients are more prone to have acute rejection events with specific alleles for SNPs rs1045642 and rs2032582 of ABCB1/MDR1. Consequently, certain allele variants by modifying immune responses or the effectiveness of the drugs may compromise the success of the immunosuppressive therapy and put patients at higher risk to reject the new organ. Therefore screening for these polymorphisms before transplantation would help clinicians to more accurately personalize medications to maximize immunosuppression and minimize toxicity.

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