

CD86 +1057G>A Polymorphism and Susceptibility to Acute Kidney Allograft Rejection

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Introduction. CD86 is a costimulatory molecule that participates in the regulation of T-cell lymphocytes activation. Thus, we examined a genetic marker on the *CD86* gene in kidney transplant outcome. **Materials and Methods.** In our retrospective study, 168 kidney allograft recipients were genotyped by direct sequencing. Patients were classified into 2 groups of 29 human leukocyte antigen (HLA)-identical haplotype allograft recipients and 139 recipients showing one or more mismatches in the HLA haplotype. Forty-five patients (26.8%) developed at least 1 acute rejection (AR) episode, 7 in the first and 38 in the second group.

Results. Acute rejection was associated with the presence anti-HLA antibodies before transplantation ($P = .03$). The AA genotype and A allele at position +1057 in the *CD86* gene were more frequent in patients without AR (9.75% and 28.5%, respectively) compared with those showing an AR (2.22% and 23.3%, respectively). This difference was statistically significant in the anti-HLA-positive recipients, as AA frequency was 31.3% in non-AR patients and zero in AR ones ($P = .04$) and A allele frequency was 46.9% and 20.8%, respectively ($P = .04$). Patients bearing AA genotype reached a higher graft survival time (9.84 years) than those carrying GA (8.21 years, $P = .32$) or GG (7.61 years, $P = .72$) genotypes.

Conclusion. These results suggest that AA genotype and A allele of *CD86* +1057G>A polymorphism may confer a protection against acute kidney allograft rejection in Tunisian patients.

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INTRODUCTION

Organ transplantation is the preferred therapy for most patients with end-stage renal disease.^{1,2} Transplantation improves both quality of life and survival.³ Unfortunately, the rate of kidney allograft rejection remains important. With the aim of reducing the level of transplantation failure, several studies achieved to elucidate the immunological mechanisms involved in this process. Thus, it was well established that allograft rejection is an

immune response strongly depending on T cells proliferation. In fact, T-cell lymphocytes play a crucial role in the initiation and the regulation of the adaptive immune response to foreign or native antigens.⁴

The activation of naive T cells requires 2 distinct signals. The first one is mediated by the association of the T-cell receptor with the major histocompatibility complex molecules, known in humans as the human leukocyte antigen (HLA),⁵ and

the second is provided by the interaction between CD28 on the T-cell surface and CD86 or CD80 on the antigen presenting cells.^{6,7} This costimulatory signal leads to clonal T lymphocytes expansion and differentiation and to cytokines expression.^{8,9} In a later step, T lymphocytes proliferation is downregulated by the binding of CD80 and CD86 to the cytotoxic T lymphocyte antigen-4, the counter receptor of the CD28, also expressed on the T-cell surface.^{10,11} This interaction inhibits immune response and may induce immune tolerance, which is fundamental for allograft acceptance.¹² Thus, the T-cell costimulatory activation pathway may have an important influence on transplantation outcome,^{13,14} and it is interesting to examine the genetic polymorphisms of the molecules involved in this process.

In this study, we focused on a G-to-A transition (a single nucleotide polymorphism) at position +1057 of the *CD86* gene. This polymorphism results in an alanine-threonine substitution at codon 304 located in the cytoplasmic tail of the CD86 molecule and could lead a potential phosphorylation site in this region, which may influence the antigen presenting cells-signal transduction pathway induced by CD86 and modify their capacity to induce graft alloresponse.¹⁵ Thus, we analyzed the association of the G-to-A substitution at nucleotide +1057 (+1057G>A polymorphism) in the *CD86* gene with susceptibility of acute rejection (AR) episodes in Tunisian kidney allograft recipients.

MATERIALS AND METHODS

Patients

We retrospectively investigated the genotypic distribution of the *CD86* +1057G>A polymorphism in 168 kidney transplant recipients. These patients were classified into 2 groups according to the HLA-haplotype similarity between donor and recipient: Group 1 included 29 HLA-identical haplotype allograft recipients, and group 2 included 139 recipients showing one or more mismatches in the HLA haplotype. The AR diagnosis was made by clinical, histological, and biochemical standard assessment (Banff criteria). All patients, except those of group 1, received rabbit antithymocyte globulin as induction therapy. Antithymocyte globulin was also administrated after an AR episode in 13 patients of group 1 and 8 of group 2. As maintenance immunosuppression, all

patients received prednisolone, tacrolimus, and/or mycophenolate mofetil (Table 1). Prior to transplantation, the sera of 166 patients (29 from group 1 and 137 from group 2) were tested for HLA antibodies by the microlymphocytotoxicity assay.

Methods

DNA Extraction. Genomic DNA was extracted from peripheral blood leukocytes according to the “salting out” standard method.

***CD86* +1057G>A Polymorphism Genotyping.** For the amplification of a DNA fragment containing the +1057G>A polymorphism on the exon 8 of the *CD86* gene, a polymerase chain reaction (PCR) was performed using a PCR System 2700 Thermal Cycler (GeneAmp, Applied Biosystems, Foster City, CA, USA). Primers used were as follows¹⁵:

Forward Ex 8/3:

5'-CTCCTCATTGCTGTTCCAATGGCAACC-3'

Reverse Ex 8/4:

5'-CATGAGCCATTAAGCTGGGCTTGGCCC-3'

The PCR protocol was as follows: a final volume of 20 μ L, containing 50 ng of genomic DNA, 1.5 mM of magnesium chloride, 0.2 mM of dNTP, 10 pmol of each primer, and 0.5 U of Taq DNA polymerase (Promega, Madison, WI, USA). The DNA was denatured at 94°C for 4 minutes, prior to 30 cycles of amplification. The conditions used for each cycle were denaturation for 30 seconds at 94°C, annealing for 30 seconds at 67°C, and extension for 1 minute at 72°C. The 30 amplification cycles were followed by a final extension step at 72°C for 5 minutes. The PCR products were resolved in 2% agarose gels stained with ethidium bromide. The amplified fragments were then sequenced in forward direction using the Ex 8/3 primer. Sequencing was performed with 100 ng of DNA using ABI PRISM Dye Terminator Cycle Ready Reaction kit (Applied Biosystems, Foster City, USA) under recommended conditions. Sequenced samples were purified using Centri-Sep columns (Dye EXTm 2.0 Spin Kit, Qiagen, Valencia, CA, USA) according to manufacturer's instructions, loaded in a PE ABI Prisms 310 Genetic Analyzer (Perkin Elmer, Waltham, MA, USA) and analyzed using ABI Prisms Navigator Software. The two alleles G and A at position +1057 were observed as different fluorescence peaks in that position (Figure 1).

Table 1. Epidemiological Features of Kidney Allograft Recipients

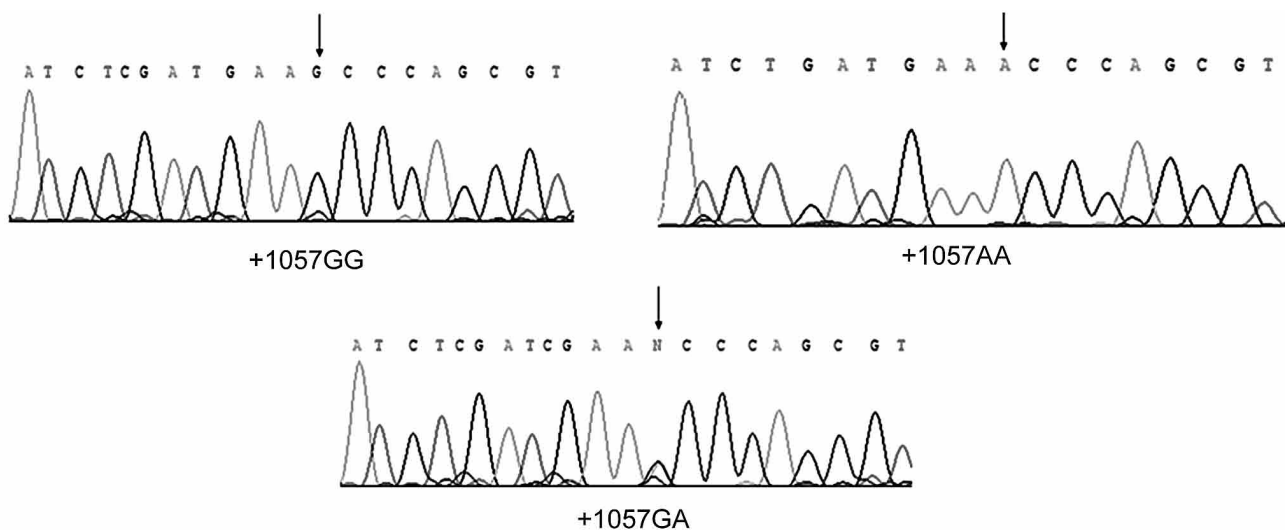
Features	Patients	Group 1	Group 2
Age, y	32.08 ± 10.92	31.74 ± 6.70	32.36 ± 11.51
Gender			
Male	103	22	81
Female	65	7	58
Donor source			
Related living	128 (76.2)	29 (100)	99 (71.22)
Unrelated living	35 (20.8)	0	35 (25.18)
Decreased	5 (3.0)	0	5 (3.60)
Follow-up, mo	85.0 ± 63.6	129.0 ± 73.8	75.0 ± 57.1
Initial nephropathy			
Glomerulonephritis	64	17	47
Unspecified chronic nephropathy	53	3	50
Tubulointerstitial nephropathy	32	6	26
Vascular nephropathy	12	2	10
Rapidly evolutionary nephropathy	4	1	3
Gravidic nephropathy	1	0	1
Hereditary nephropathy	1	0	1
Henoch-Schonlein purpura	1	0	1
Immunosuppressive treatment			
Double regimen			
PRED+AZA	22 (13.0)	13 (44.8)	9 (9.3)
PRED+MMF	21 (12.5)	5 (17.2)	16 (11.5)
PRED+CSA	1 (0.6)	0	1 (0.7)
Triple regimen			
PRED+CSA+AZA	34 (20.2)	4 (13.8)	28 (20.1)
PRED+CSA+MMF	74 (44.0)	5 (17.24)	69 (49.6)
PRED+MMF+TAC	16 (9.5)	2 (8.62)	14 (10.1)
Acute rejection episode	45 (26.8)	7 (24.14)	38 (27.3)
Pretransplant anti-HLA antibodies	28	4	24
Acute rejection patients	12 (42.9) [†]	2 (50.0) [‡]	10 (41.7) [§]
Non-acute rejection patients	16 (57.1) [†]	2 (50.0) [‡]	14 (58.3) [§]

*Data are given as mean standard deviation and absolute number (percent) where appropriate. PRED indicates prednisolone; AZA, azathioprine; MMF, mycophenolate mofetil; CSA, cyclosporine A; and TAC, tacrolimus.

[†] $P = .03$; odds ratio, 2.48; 95% confidence interval, 0.98 to 6.27

[‡] $P = .23$

[§] $P = .12$

**Figure 1.** Sequence profiles of CD86 +1057GA genotypes.

Statistical Analyses

Statistical comparisons were performed between patients and controls by the Pearson chi² test calculated on 2-by-2 contingency tables. The Fisher exact test was used when an expected cell value was less than 5. The strength of the association between genotypes or alleles in each group was estimated by the calculation of the odds ratios (OR) and 95% confidence intervals (CI). Survival rates were evaluated by the StatView software (SAS, Cary, NC, USA). A *P* value less than .05 was considered significant.

RESULTS

Patient Characteristics

No significant differences were found between groups of patients in gender, age, ethnicity, or primary disease (Table 1). Forty-five patients (26.8%) developed at least 1 AR episode within the first 6 months after transplantation. Since the major histocompatibility complex molecules play a crucial role in the mechanism of allograft acceptance,^{13,16-18} we classified the patients into 2 groups according to HLA compatibility between graft donor and graft recipient. As shown in Table 1, the rate of AR was lower in group 1 (24.1%) than in group 2 (27.3%) or in the global group of patients (26.8%).

Before transplantation, 28 of 166 kidney recipients were anti-HLA positive. They were 4 of 29 patients in group 1, two of whom developed an AR episode, and 24 of 137 in group 2, including 10 with an AR episode. Among the 138 anti-HLA-negative recipients, 25 were in group 1 including 5 with an AR episode and 113 were in group 2, including 27 showing an AR episode. The incidence of rejection was significantly associated with the presence of HLA antibodies before transplantation in the 166 patients (*P* = .03; Table 1).

CD86 +1057G>A Polymorphism and Acute Rejection

There was no departure from Hardy-Weinberg equilibrium in patients groups. The distribution of *CD86* genotypes and alleles frequencies was similar in the patients of group 1 and group 2.

The frequency of AA genotype of the *CD86* +1057G>A polymorphism was higher in the patients without AR (9.7%) than in those with an AR episode (2.2%). However, this difference is not statistically significant (*P* = .10). In group 2, the AA genotype was absent in the 38 patients with AR (Table 2). In fact, there was a trend towards an increase of the A allele frequency among the patients who did not developed an AR allograft, compared to the kidney recipients with AR (28.5% versus 23.3% in the whole group of patients, *P* = .34; 31.8% versus 21.4% in group 1, *P* = .52; and 27.8% versus 23.7% in group 2, *P* = .46; Table 2).

As shown in Table 3, it was noticed that the frequency of AA genotype in anti-HLA-positives was significantly higher in non-AR patients than in AR ones (31.3% versus zero, *P* = .03; OR, 11.95; 95% CI, 0.59 to 241). A similar association between a decrease of AR and A allele frequency was observed in this group (20.8% in AR and 46.9% in non-AR, *P* = .04; OR, 0.29; 95% CI, 0.08 to 0.99). The statistical power (1- beta) was 35%.

CD86 +1057G>A Polymorphism and Graft Survival

After 10 years of follow-up, the AA-bearing recipients reached a higher mean graft survival time (9.84 years) than those carrying GA (8.21 years, *P* = .32) or GG (7.61 years, *P* = .72) genotypes (Figure 2). Nevertheless, these differences were not significant.

Table 2. *CD86* Genotype and Allele Frequencies in Kidney Transplant Recipients*

Genetic Parameters	All Patients		Group 1		Group 2	
	AR (n = 45)	Non-AR (n = 123)	AR (n = 7)	Non-AR (n = 22)	AR (n = 38)	Non-AR (n = 101)
Genotype						
GG	25 (55.6)	65 (52.84)	5 (71.4)	11 (50.0)	20 (52.6)	54 (53.5)
GA	19 (42.2)	46 (37.4)	1 (14.3)	8 (36.4)	18 (47.4)	38 (37.6)
AA	1 (2.2)	12 (9.7)	1 (14.23)	3 (13.6)	0	9 (8.9)
Allele						
G	69 (76.7)	176 (71.5)	11 (78.6)	30 (68.2)	58 (76.3)	146 (72.2)
A	21 (23.3)	70 (28.5)	3 (21.4)	14 (31.8)	18 (23.7)	56 (27.8)

*Values in parentheses are percents. AR indicates acute rejection.

Table 3. *CD86* Genotype and Allele Frequencies in Anti-Human Leukocyte Antigen (HLA)-Positive and Anti-HLA Negative Kidney Transplant Recipients With and Without Acute Rejection (AR)

Subgroups	Patients	Genotypes (%)			Alleles, %	
		GG	GA	AA	G	A
Anti-HLA+	28	13 (46.4)	10 (31.7)	5 (17.9)	64.3	35.7
Anti-HLA-	138	76 (55.1)	54 (39.1)	8 (5.8)	74.6	25.4
Anti-HLA+						
AR	12	7 (58.3)	5 (41.7)	0*	79.2	20.8†
Non-AR	16	6 (37.5)	5 (31.3)	5 (31.2)*	53.1	46.9†
Anti-HLA-						
AR	32	17 (53.1)	14 (43.8)	1 (3.1)	75.0	25.0
Non-AR	106	59 (55.7)	40 (37.7)	7 (6.6)	74.5	25.5
Anti-HLA+						
Group 1	4	3 (75.0)	0	1 (25.0)	75.0	25.0
Group 2	24	10 (41.7)	10 (41.7)	4 (16.7)	62.5	37.5
Anti-HLA-						
Group 1	25	13 (52.0)	9 (36.0)	3 (12.0)	70.0	30.0
Group 2	113	63 (55.7)	45 (39.8)	5 (4.4)	75.6	24.4
Anti-HLA+						
Group 1						
AR	2	2 (100)	0	0	100	0
Non-AR	2	1 (50.0)	0	1 (50.0)	50.0	50.0
Group 2						
AR	10	5 (50.0)	5 (50.0)	0	75.0	25.0
Non-AR	14	5 (31.7)	5 (31.7)	4 (28.6)	53.6	46.4
Anti-HLA-						
Group 1						
AR	5	3 (60.0)	1 (20.0)	1 (20.0)	70.0	30.0
Non-AR	20	10 (50.0)	8 (40.0)	2 (10.0)	70.0	30.0
Group 2						
AR	27	14 (51.9)	13 (48.1)	0	75.9	24.1
Non-AR	86	49 (57.0)	32 (37.2)	5 (5.8)	75.6	24.4

* $P = .03$; odds ratio, 11.95; 95% confidence interval, 0.59 to 241

† $P = .04$; odds ratio, 0.29; 95% confidence interval, 0.08 to 0.99

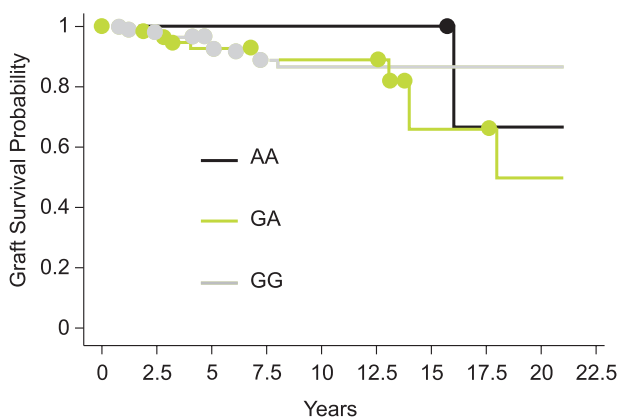


Figure 2. Actuarial analysis of kidney allograft survival rates according to the *CD86* +1057G>A polymorphism.

DISCUSSION

Our findings suggest that +1057AA genotype or A allele of *CD86* gene may have a protective

effect against AR in kidney allografts. In fact, the A allele was more frequent in non-AR recipients of the global group of HLA-identical haplotype allograft recipients (group 1) and the recipients showing one or more mismatches in the HLA haplotype (group 2), as compared to AR recipients. The AA genotype frequency was also higher in the patients without AR than in those with at least one AR episode. In group 2, patients carrying the AA genotype did not show any AR, but it is important to note that allograft rejection in this group was due above all to the HLA disparity between donors and recipients. In group 1, AA incidence was a little lower in non-AR than in AR patients, but that can be explained by the small proportion of AR patients in this group (7 patients). In addition, it is established that preformed anti-HLA antibodies in allograft recipients can induce severe vascular disease of organ transplant.¹⁹⁻²¹ In

our study, sensitized patients were more susceptible to develop AR than nonsensitized ones. We also noticed a significant increase of the AA genotype and A allele frequencies in anti-HLA-positive non-AR recipients than in AR ones. Thus, we can presume that the simultaneous presence of anti-HLA antibodies and of the +1057G allele may generate cumulate effects and enhance the risk of AR in kidney transplant recipients.

Furthermore, the mean graft survival time after 10 years of follow-up was higher in AA carrying recipients than in GA or GG ones. Even though this comparison did not show any significant difference, we can suppose that AA genotype procures a better long-term graft survival time to kidney recipients than GG or GA genotypes.

The wide CIs in this analysis reflect the relatively small numbers in some of the subgroups, with a consequent loss of statistical power. A larger patient cohort would therefore be required for us to reduce the width of the CIs and confirm this observation. More studies are needed in order to clarify if determination of the patients *CD86* genotypes could be a useful prognostic factor in kidney allograft failure.

Taken together, our results join those of Marin and colleagues, who suggested that +1057AA genotype and A allele of the *CD86* gene might reduce allograft rejection incidence in liver transplantation.¹⁵ Minguela and colleagues also analyzed the expression of costimulatory molecules in liver transplant and demonstrated that upregulation of CD28/cytotoxic T lymphocyte antigen-4/*CD86* molecules was associated with AR of liver allograft.¹² Furthermore, previous studies have shown that the blockade of CD28-CD86 binding prolongs graft survival and induces specific tolerance.²²⁻²⁴ It is also established that CD86 molecule is involved in the triggering of human natural killer cells.²⁶ Therefore, the gene of the CD86 molecule was a candidate for a large spectrum of autoimmune diseases such as rheumatoid arthritis, asthma, and systemic lupus erythematosus.²⁵⁻²⁷

CONCLUSIONS

The analysis of the *CD86* +1057G>A polymorphism and its association with AR episodes in our population showed that the frequencies of AA genotype and A allele were higher in non-AR than in AR patients, indicating that the presence

of AA genotype or A allele in kidney recipients may have a protective effect against AR incidence and may confer an increase of long-term kidney graft survival. However, it should be of interest to confirm our findings in a larger series of patients since the frequency of AA genotype is relatively low. It is also important to examine other costimulatory molecules which interfere in allograft acceptance.

CONFLICT OF INTEREST

None declared.

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