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ORIGINAL RESEARCH

Tumor Necrosis Factor-α Gene Polymorphism is Associated with Short- and Long-Term Kidney Allograft Outcomes

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Introduction: Kidney transplantation has excellent short-term results with current immunosuppression regimes, but long-term outcomes have barely improved over the past two decades. Hence, there is a need for new therapeutic options to increase long-term survival of kidney grafts. Drug development for kidney transplantation has slowly plateaued, limiting progress while making drug repurposing an attractive alternative. We, therefore, investigated the impact of tumor necrosis factor-alpha (TNF-α) gene (*TNF*) polymorphisms on kidney graft survival after transplantation.

Methods: We performed a prospective cohort study to assess the association of *TNF* polymorphisms (rs1800629 G>A and rs3093662 A>G) with primary non-function and death-censored kidney allograft survival in 1271 kidney transplant pairs from the University Medical Center Groningen in The Netherlands.

Results: The G-allele of the *TNF* rs3093662 polymorphism in donor kidneys was associated with a higher risk of immediate graft loss (odds ratio: 2.05; 95%-CI: 1.06–3.97; $P = 0.032$). Furthermore, the G-allele of this *TNF* rs3093662 polymorphism in the donor was also associated with worse 5-year, 10-year, and 15-year death-censored kidney graft survival ($P < 0.05$). The cumulative incidence of graft loss was 15.9% in the reference AA-genotype group and 25.2% in the AG/GG-genotype group, respectively. In multivariable analysis, the association between the *TNF* rs3093662 polymorphism in the donor and 15-year death-censored kidney graft survival remained significant (hazard ratio: 1.51; 95%-CI: 1.05–2.19, $P = 0.028$).

Discussion: In conclusion, kidney allografts possessing a high-producing *TNF* polymorphism have a greater risk of immediate and late graft loss. Our study adds to a growing body of literature indicating the potential of TNF-α blockade in improving kidney transplantation outcomes.

Keywords: cytokines, kidney transplantation, nephrology, genetics

Introduction

Kidney transplantation has excellent short-term outcomes with current immunosuppression regimes, while long-term allograft survival has barely improved over the past decades.[1] Consequently, there is a need for new therapies that can increase the long-term survival of kidney grafts in transplant recipients. Drug development in nephrology and transplantation has been slow and limited in the past twenty years for a multitude of reasons, most notable of which are the steep costs of developing new drugs and identifying and enrolling the patients for clinical trials.[2,3] Multi-disciplinary efforts are now underway to accelerate the process of drug development for renal transplant patients.[3,4] Simultaneously, drug repurposing is seen as an attractive alternative to the traditional drug discovery process.[5] With drug repurposing investigators explore new indications of use for investigational or previously approved drugs. Notably, this approach

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is low-cost, time-saving, risk-averse, and, therefore, highly efficient.[5] Recycling existing anti-inflammatory drugs for kidney transplantation, thus, should be considered to improve long-term transplant outcomes.

Cytokines induce and enhance the immune response to combat invading microorganisms, but can also cause severe host injury or disease if the activation of this inflammatory response is excessive or unwanted.[6] Among all cytokines, tumor necrosis factor-alpha (TNF-α) continues to be one of the most extensively studied.[7] Currently, there are five therapeutic agents in clinical use that target TNF-α; Four monoclonal antibodies (mAb; Infliximab, Centolizumab, Adalimumab, and Golimumab) and one fusion protein (Etanercept).[8] The clinical success of TNF-α targeted therapies in rheumatologic disorders and inflammatory bowel diseases has helped to establish these biologicals as the mainstay treatment for autoimmune diseases.[9,10] Currently, there is substantial evidence that TNF-α plays an equally important role in renal inflammation and fibrosis.[11] Several studies have also demonstrated the importance of TNF-α in renal ischemia-reperfusion injury by using a variety of TNF-α blocking strategies.[12–14] On this basis, TNF-α inhibitors have therefore been proposed for use in kidney transplant recipients to improve outcome.[15]

Here, we used human genetics to probe whether targeting TNF-α in renal transplantation will provide clinical benefit. Studies of human genetics are powerful tools in validating therapeutic targets because genetically-backed drug targets are reported to be twice as likely to lead to approved therapeutics compared to traditional drug discovery targets.[16,17] Hence, we investigated the association between functional TNF-α gene (*TNF*) polymorphisms and kidney graft survival in donor-recipient renal transplant pairs. The two common single nucleotide polymorphisms (SNPs) of the TNF-α gene we studied here are the rs1800629 G>A in the promoter region at position ~308 and rs3093662 A>G in the first intron at position ~851 (Figure 1). The A-allele of the rs1800629 SNP A allele leads to a higher transcriptional activity and is often connected to autoimmune diseases, while the G-allele of the rs3093662 SNP is associated with higher serum levels of TNF-α.[18,19]

Materials and Methods

Patient Selection and Study End-Point

Patients were enrolled if they underwent single kidney transplantation between March 1993 and February 2008 at the University Medical Center Groningen (UMCG) in the Netherlands. A total of 1430 kidney transplantations were screened for analysis. Of these, 1271 donor and recipient kidney transplant pairs were included in the study as previously described.[20–24] Exclusion criteria included lack of DNA, technical complications during surgery, loss of follow-up or re-transplantation. The primary endpoint of our study was primary non-function (PNF) and long-term death-censored graft survival, defined as the need for re-transplantation or dialysis. Post-transplantation, patients were followed-up and immunosuppression regimen, laboratory and clinical parameters, and time to graft loss were documented. Donor and recipient characteristics, transplantation-related parameters and clinical data were retrieved from medical records.

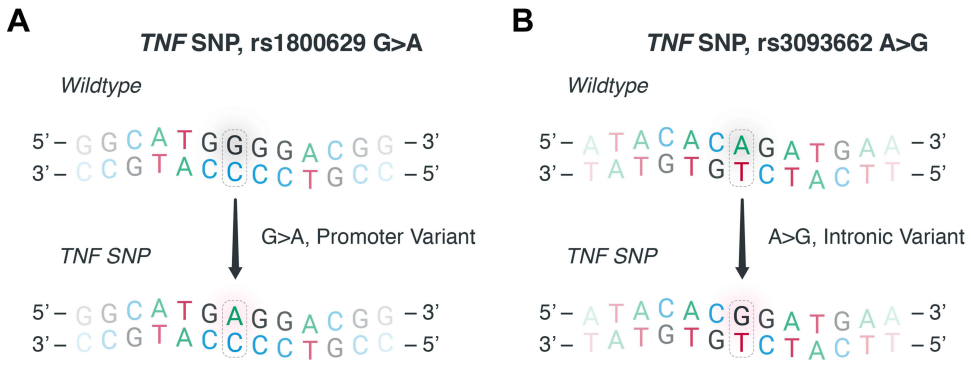

Figure 1 Examined polymorphisms in the tumor necrosis factor-alpha gene. To assess the impact of single nucleotide polymorphisms (SNP) in the tumor necrosis factor-alpha (TNF-α) gene (*TNF*) in kidney transplantation, we assessed the association between graft survival outcomes and the (**A**) *TNF* SNP rs1800629 G>A and the (**B**) *TNF* SNP rs3093662 A>G in the donors and recipients. The *TNF* SNP rs1800629 G>A is located in the promoter region while the *TNF* SNP rs3093662 A>G is in the first intron of the gene.

DNA Extraction and *TNF* Genotyping

Blood was obtained for the isolation of peripheral blood mononuclear cells or splenocytes were collected from the donors and recipients. DNA isolation of samples was performed with the Qiagen FlexiGene® DNA AGF3000 Kit on an AutoGenFlex 3000 workstation (Autogen, Holliston, MA, USA), according to the manufacturer's instructions and stored at −80°C. Absorbance at 260 nm was analyzed with a spectrophotometer (ND-1000, NanoDrop Technologies), and DNA concentration was assessed by the NanoDrop nucleic acid application module. As an indicator of DNA purity, 260/230 and 260/280 absorbance ratios were calculated. Where samples failed to meet the recommended purity or the minimum DNA concentration for Illumina genotyping, isolation attempts were repeated. Genotyping of single nucleotide polymorphisms (SNPs) was determined using the Illumina VeraCode GoldenGate Assay kit as per the manufacturer's instructions (Illumina, San Diego, CA, USA). The promoter of *TNF* contains several SNPs, of which the rs1800629 G>A has been most extensively studied.[25] In addition, we chose the *TNF* rs3093662 A>G SNP, which has previously shown to increase systemic levels of TNF-α and influence graft function after kidney transplantation.[18,26] The selected SNPs were not in linkage disequilibrium ($r^2 < 0.10$; SNP Annotation and Proxy Search, Broad institute). Genotype clustering and calling were performed using BeadStudio Software (Illumina). The overall genotype success rate was 99.5% and 6 samples with a high missing call rate were excluded from subsequent analyses.

Statistical Analysis

SPSS software version 25 (SPSS Inc, Chicago, IL, USA) was used to perform statistical analyses. Data are presented as median [IQR] for non-parametric variables, mean ± standard deviation for parametric variables, and nominal variables as the total number of patients with percentage [n (%)]. Groups were assessed for differences with the Mann–Whitney *U*-test for not-normally divided variables or Student *t*-test for normally distributed variables, and χ^2 test for categorical variables, respectively. The Log rank test was used to determine the difference in the graft loss frequency between different genotypes. Univariable analysis was used to assess the association of genetic, recipient, donor, and transplant characteristics with graft loss. The variables identified in these analyses were next examined in a multivariable Cox regression with a stepwise forward selection. Statistical testing was 2-tailed and $P<0.05$ was regarded as significant.

Results

Population Characteristics

The characteristics of all donor and recipient kidney transplant pairs are shown in Table 1. The maximum study period was 15 years, with a mean follow-up after transplantation of 6.16 years ± 4.21. During the study period, 214 grafts (17.4%) were lost with the main causes of graft loss being rejection (n = 126), surgical complications (n = 33), recurrence of primary disease (n = 16), vascular causes (n = 12), other causes (n = 16) and unknown (n = 11). By univariable analysis, recipient age, donor age, donor type (living vs cadaveric), recipient blood type (AB vs others), donor blood type (AB vs others), cold ischemia time, warm ischemia time, use of corticosteroids, use of cyclosporin, and delayed graft function (DGF) were found to be associated with graft loss ($P < 0.05$).

The observed genotypic frequencies of the *TNF* SNP rs1800629 G>A were significantly different between recipients (n = 1266; GG, 58.3%; GA, 34.3%; AA, 7.0%) and donors (n = 1269; GG, 68.1%; GA, 29.0%; AA, 2.7%) ($P < 0.001$). More specifically, the A-allele of the *TNF* rs1800629 SNP was more prevalent in kidney transplant recipients. The frequency of the *TNF* rs1800629-A variant were also significantly higher in both donors and recipients than those reported by the 1000 genomes project ($P < 0.001$).[27] The observed genotypic frequencies of the *TNF* rs3093662 A>G was not significantly different between recipients (n = 1270; AA, 89.3%; GA, 10.6%) and donors (n = 1267; AA, 88.4%; AG, 10.9%; GG, 0.4%) ($P = 0.08$). Yet, the frequency of the *TNF* rs3093662-G variant described in the 1000 genomes project was significantly higher than those in the donors ($P = 0.003$) and recipients ($P < 0.001$).[27] Due to the low frequency in donors of the *TNF* rs3093662 polymorphism GG-genotype, heterozygotes (AG) and homozygotes (GG)-genotypes were combined to one group (AG/GG). Lastly, the distribution of both polymorphisms was in Hardy−Weinberg equilibrium.

Table 1 Baseline Characteristics of the Donors and Recipients

		All Patients (n = 1271)	Functioning Graft (n = 1056)	Graft Loss (n = 215)	P-value*	HR	P-value#
Recipient							
***TNF* rs3093662 SNP**	AA, n (%)	1135 (89.4)	940 (89.1)	195 (90.7)	0.49		0.52
	AG, n (%)	135 (10.6)	115 (10.9)	20 (9.3)			
***TNF* rs1800629 SNP**	GG, n (%)	741 (58.5)	619 (58.8)	122 (57.3)	0.69		0.80
	GA, n (%)	436 (34.4)	358 (34.0)	78 (36.6)			
	AA, n (%)	89 (7.0)	76 (7.2)	13 (6.1)			
Female sex, n (%)		532 (41.9)	449 (42.5)	83 (38.6)	0.29		0.21
Age, years		47.9 ± 13.5	48.5 ± 13.4	45.0 ± 13.2	**<0.001**	0.99	**0.027**
Dialysis vintage, weeks		172 [91–263]	174 [87–261]	168 [109–270]	0.15		0.10
Blood group donor							
Type O, n (%)		567 (44.6)	474 (44.9)	93 (43.3)	**0.004**	0.46	**0.002**
Type A, n (%)		536 (42.2)	448 (42.4)	88 (40.9)		0.46	**0.002**
Type B, n (%)		113 (8.9)	98 (9.3)	15 (7.0)		0.35	**0.002**
Type AB, n (%)		55 (4.3)	36 (3.4)	19 (8.8)		Ref	**0.008**
Primary kidney disease							
Glomerulonephritis, n (%)		340 (26.8)	271 (25.7)	69 (32.1)	0.32		0.45
Polycystic disease, n (%)		208 (16.4)	187 (17.7)	21 (9.8)			
Vascular disease, n (%)		145 (11.4)	122 (11.6)	23 (10.7)			
Pyelonephritis, n (%)		148 (11.6)	120 (11.4)	28 (13.0)			
Diabetes, n (%)		51 (4.0)	44 (4.2)	7 (3.3)			
Chronic, n (%)		168 (13.2)	134 (12.7)	34 (15.9)			
Other, n (%)		211 (16.6)	178 (16.9)	33 (15.3)			
Immunosuppression							
Anti-CD3 Moab, n (%)		19 (1.5)	14 (1.3)	5 (2.3)	0.27		0.51
ATG, n (%)		103 (8.1)	79 (7.5)	24 (11.2)	0.07		0.14
Azathioprine, n (%)		72 (5.7)	53 (5.0)	19 (8.8)	**0.027**		0.29
Corticosteroids, n (%)		1201 (94.5)	1002 (94.9)	199 (92.6)	0.17	0.51	**0.01**
Cyclosporin, n (%)		1085 (85.4)	911 (86.3)	174 (80.9)	**0.044**	0.66	**0.016**
Interleukin-2 RA, n (%)		199 (15.7)	163 (15.4)	36 (16.7)	0.63		0.12
Mycophenolic acid, n (%)		907 (71.4)	775 (73.4)	132 (61.4)	**<0.001**		0.06
Sirolimus, n (%)		38 (3.0)	33 (3.1)	5 (2.3)	0.53		0.54
Tacrolimus, n (%)		97 (7.6)	77 (7.3)	20 (9.3)	0.31		0.39

(*Continued*)

Table 1 (Continued).

		All Patients (n = 1271)	Functioning Graft (n = 1056)	Graft Loss (n = 215)	P-value*	HR	P-value#
Donor							
***TNF* rs3093662 SNP**	AA, n (%)	1124 (88.7)	945 (89.8)	179 (83.3)	**0.006**	Ref	**0.005**
	AG/GG, n (%)	143 (11.3)	107 (10.2)	36 (16.7)		1.67	
***TNF* rs1800629 SNP**	GG, n (%)	866 (68.2)	714 (67.7)	152 (70.7)	0.66		0.57
	GA, n (%)	369 (29.1)	312 (29.6)	57 (26.5)			
	AA, n (%)	34 (2.7)	28 (2.7)	6 (2.8)			
Female sex, n (%)		626 (49.3)	521 (49.3)	105 (48.8)	0.89		0.96
Age, years		44.4 ± 14.4	44.1 ± 14.6	46.1 ± 13.4	**0.044**	1.02	**<0.001**
Donor type							
Living, n (%)		282 (22.2)	257 (24.3)	25 (11.6)	**<0.001**	Ref	**0.002**
Brain death, n (%)		787 (61.9)	642 (60.8)	145 (67.4)		1.94	
Circulatory death, n (%)		202 (15.9)	157 (14.9)	45 (20.9)			
Blood group							
Type O, n (%)		642 (50.6)	541 (51.3)	101 (47.2)	**0.033**	0.39	**0.004**
Type A, n (%)		502 (39.6)	414 (39.3)	88 (41.1)		0.42	**0.01**
Type B, n (%)		97 (7.6)	82 (7.8)	15 (7.0)		0.36	**0.012**
Type AB, n (%)		27 (2.1)	17 (1.6)	10 (4.7)		Ref	**0.035**
Transplantation							
Highest PRA, in %		10.1 ± 23.6	9.98 ± 23.7	10.9 ± 25.0	0.54		0.75
Total HLA mismatches		2 [1–3]	2 [1–3]	2 [1–3]	0.48		0.11
CIT, in hours		17.7 [10.9–23.0]	17.0 [8.6–23.0]	20,0 [15.3–25.0]	**<0.001**	1.03	**0.001**
WIT, in minutes		37.0 [31–45]	37.0 [30–45]	38.0 [32–45]	0.12	1.02	**0.003**
DGF, n (%)		415 (32.7)	289 (27.4)	126 (58.6)	**<0.001**	3.79	**<0.001**

Notes: The characteristics of all donor and recipient kidney transplant pairs as well as subgroup analysis for graft loss after 15 years of follow-up. Data are displayed as the total number of patients with percentage for nominal data [n (%)]; median [IQR] for non-parametric variables, and; mean ± standard deviation for parametric variables. Bold *P-values* indicate *P-values* that are statistically significant (*P*-value < 0.05). *P-value** shows the P-value for the differences in baseline demographics among the groups, tested by χ^2 test for categorical variables, and Mann–Whitney *U*-test or Student's *t*-Test for continuous variables. *P-value#* shows the P-value for univariable analysis for 15-year death censored graft survival.

Abbreviations: ATG, anti-thymocyte globulin; CD3, cluster of differentiation CIT, cold ischemia time; DGF, delayed graft function; HLA, human leukocyte antigen; PRA, panel-reactive antibody; 3; RA, receptor antagonist; SNP, single nucleotide polymorphism; *TNF*, tumor necrosis factor-alpha gene; WIT, warm ischemia time.

A Genetic Variant in *TNF* is a Risk Factor for Early Graft Loss After Kidney Transplantation

We first studied whether genetic variants in *TNF* are associated with PNF to assess the impact on early graft loss. For the *TNF* rs3093662 A>G polymorphism, the proportion of grafts with PNF significantly differed based on the donor genotype (4.3% PNF in the AA-genotype group vs 8.4% PNF in the AG/GG-group, $P = 0.029$), but not for recipient genotype ($P = 0.31$) (Table 2). In univariable logistic regression, donors carrying the G-allele of the *TNF* rs3093662 A>G

Table 2 Genotype Frequency and Hazard Ratios for Primary Non-Function Based on TNF Genotypes

TNF rs3093662 SNP	**Primary Non-Function**		**Immediate Graft Function**		***P*-value[a]**	**Odds Ratio (95%-CI)**	***P*-value[b]**
	AA	**AG/GG**	**AA**	**AG/GG**			
Donor	48 (80.0%)	12 (20.0%)	1076 (89.1%)	131 (10.9%)	**0.029**	**2.05** (1.06–3.97)	**0.032**
Recipient	56 (93.3%)	4 (6.7%)	1079 (89.2%)	56 (4.9%)	0.31		0.32

Notes: Differences in the occurrence of primary non-function (PNF) based on donor and recipient tumor necrosis factor-alpha genotypes. Bold *P-values* indicate *P-values* that are statistically significant (*P*-value < 0.05). [a]P-value for the Pearson Chi-square test for differences in genotype frequency. [b]P-value for univariate logistic regression analysis for differences in the incidence of primary non-function.

Abbreviations: 95-CI%, 95% confidence interval; SNP, single nucleotide polymorphism; *TNF*, recipient tumor necrosis factor-alpha gene.

polymorphism had a significantly higher risk of PNF (OR = 2.05 compared to A-allele; 95%-CI: 1.06–3.97; $P = 0.032$). Next, we performed a multivariable logistic regression analysis with a stepwise forward selection procedure using all characteristics that were significantly associated with PNF in univariable logistic regression (Table 3). In the final model, the *TNF* rs3093662 A>G polymorphism in the donor, donor age, donor type (living vs cadaveric), recipient sex, recipient blood type (AB vs others), and warm ischemia time were included. After adjustment, the G-allele of rs3093662 in the donor was significantly associated with graft loss with an odds ratio of 2.16 (95% CI: 1.07–4.37, $P = 0.032$). For the other *TNF* polymorphism, namely rs1800629 G>A, the proportion of grafts with PNF did not significantly differ based on the recipient genotype ($P = 0.40$), or donor genotype ($P = 0.35$). In conclusion, our data show that the minor allele of the *TNF* rs3093662 A>G SNP in the donor associates with a higher risk of immediate graft loss after kidney transplantation.

A Genetic Variant in *TNF* is a Risk Factor for Late Graft Loss After Kidney Transplantation

Next, we studied whether the genetic variants in *TNF* are associated with kidney allograft survival to evaluate the relationship between these polymorphisms and late graft loss. No association was found between 15-year death-censored kidney graft survival and the *TNF* rs1800629 G>A polymorphism in the donor (Supplementary Figure 1, $P = 0.64$) or the recipient (Supplementary Figure 1, $P = 0.66$). Conversely, Kaplan–Meier survival analysis once again revealed that the G-allele of the *TNF* rs3093662 polymorphism in the donor was significantly associated with worse 5-year, 10-year, and 15-year death-censored kidney graft survival (Figure 2A–C). After 15 years of follow-up, the cumulative incidence of graft loss was 15.9% in the reference AA-genotype group and 25.2% in the AG/GG-genotype group, respectively. The association of the G-allele with late graft loss was maintained when PNF cases were excluded (Figure 2D, $P = 0.047$), thereby demonstrating that the association between the *TNF* rs3093662 polymorphism and long-term graft survival is independent of its association with early graft loss. We next performed a subgroup analysis for the donor type, since deceased organ donors have elevated levels of circulating pro-inflammatory cytokines. Kaplan-Meier curves demonstrated that the association remained significant between the *TNF* rs3093662 polymorphism and long-term graft survival in kidney allografts from living donors (Figure 3A, $P = 0.027$) as well as from deceased donors (Figure 3B, $P = 0.023$). Next, the donor–recipient pairs were divided into four groups based on the presence or absence of both TNF polymorphisms in the donor. Kaplan–Meier survival analyses revealed a significant difference in graft survival among the four groups (Figure 4; $P = 0.040$). Moreover, the minor allele of the *TNF* rs3093662 polymorphism in the donor seemed to have a bigger impact on graft survival than the minor allele of the *TNF* rs1800629 polymorphism. Patients receiving a renal graft carrying both minor alleles of *TNF* rs1800629 and rs3093662 polymorphisms appeared to have the worst outcome. However, this combined genotype was only identified in 34 donor–recipient pairs. The *TNF* rs3093662 polymorphism in the recipient was not associated with graft survival rates (Supplementary Figure 1, $P = 0.52$).

We additionally examined whether the G-allele of the *TNF* rs3093662 polymorphism in the donor was independently associated with long-term graft survival. In univariable analysis, the G-allele of the *TNF* rs3093662 polymorphism in the donor was associated with a hazard ratio of 1.67 (95%-CI: 1.16–2.38; $P = 0.005$) for graft loss after 15 years of follow-

Table 3 Associations of *TNF* Polymorphism in the Donor with Immediate Graft Loss After Kidney Transplantation

Variables Not in the Equation		Variables in the Equation		
Variables	**P-value**	**Variables**	**P-value**	**Hazard Ratio**
Cold ischemia time (*In hours*)	0.39	rs3093662-G in the donor (G *versus A*)	0.032	2.16 (1.07–4.37)
		Donor age *(In years)*	0.017	1.03 (1.00–1.05)
		Donor type (*Deceased versus living*)	0.002	9.53 (2.29–39.8)
		Recipient sex *(Male versus female)*	0.028	0.51 (0.28–0.93)
		Recipient blood type (*AB versus other*)	0.027	
		Warm ischemia time (*In minutes*)	0.006	1.03 (1.01–1.05)

Notes: Multivariable logistic regression was performed for primary non-function (PNF) with a stepwise forward selection. All variables with a *P*-value < 0.05 in the univariate analysis for PNF were included. Data are presented as odds ratio with 95%-confidence interval (95%-CI) and *P*-value. In the final model, the *TNF* rs3093662 A>G polymorphism in the donor, donor type, recipient sex, recipient blood type, and warm ischemia time were included, whereas cold ischemia time was not.

up. Subsequently, we performed a multivariable analysis to adjust for potential confounders using a stepwise forward selection procedure with all clinical variables that were significantly associated with kidney graft survival in univariable analysis (Table 4). In the final model, the *TNF* rs3093662 SNP in the donor, recipient and donor age, recipient blood type (AB vs others), and DGF were included. After adjustment, the G-allele of the *TNF* rs3093662 polymorphism in the donor remained significantly associated with graft loss after 15-years with a hazard ratio of 1.51 (95% CI: 1.05–2.19, $P = 0.028$). In conclusion, our results demonstrate that the minor allele of the *TNF* rs3093662 (A>G) variant in the donor associates with a higher risk of late graft loss after kidney transplantation.

Discussion

The primary finding of our study is that a donor genetic variant in *TNF* associates with an increased risk for graft loss after kidney transplantation. This *TNF* polymorphism in the donor was independently associated with both a higher risk for immediate as well as late graft loss. Extending these findings, the relationship between the *TNF* rs3093662 polymorphism and long-term kidney allograft survival remained significant in a subgroup analysis for different types of kidney transplants. In contrast, long-term graft survival was not impacted by the *TNF* rs3093662 polymorphism in the recipient, nor by the *TNF* rs1800629 polymorphism. In conclusion, our data provide genetic evidence that targeting TNF-α in renal transplantation could be favorable for long-term graft survival in kidney transplantation and encourages the study of TNF-α inhibitors in kidney transplantation in randomized controlled trials.

To our knowledge, our study is the first to show that the *TNF* rs3093662 polymorphism in the donor impacts the risk of both early and late graft loss after kidney transplantation. To summarize, we demonstrated that the G-allele in the donor nearly doubled the risk of immediate graft loss, while the relative risk of late graft loss increased by 58%. In vivo evidence regarding the functional consequences of the *TNF* rs3093662 polymorphism was recently presented, establishing the association of the G-allele with higher serum levels of TNF-α in both healthy and diseased individuals.[18] Previously, Israni et al also found an association between the *TNF* polymorphism rs3093662 G-allele in the donor and DGF after kidney transplantation.[26] Although we could not confirm this association (data not shown), the results of Israni et al are in line with our findings that this high-producing variant is detrimental in kidney allografts. Furthermore, in accordance with our study, the G-allele of the *TNF* rs3093662 polymorphism has previously been associated with increased risks of rheumatoid arthritis, adverse events in cancer patients as well as with the susceptibility to and severity of pneumonia.[18,28,29] Altogether, these studies suggest a pro-inflammatory effect of this high-producing *TNF* polymorphism, thereby increasing the risk for a wide spectrum of immune-mediated diseases.

The clinical use of TNF-α targeted therapeutics has revolutionized the management of inflammatory disorders and autoimmune diseases. In preclinical transplantation studies, TNF-α blockade showed significant protection against renal ischemia-reperfusion injury.[12–14] The underlying protective mechanisms behind targeting TNF-α include: (i) Modulating

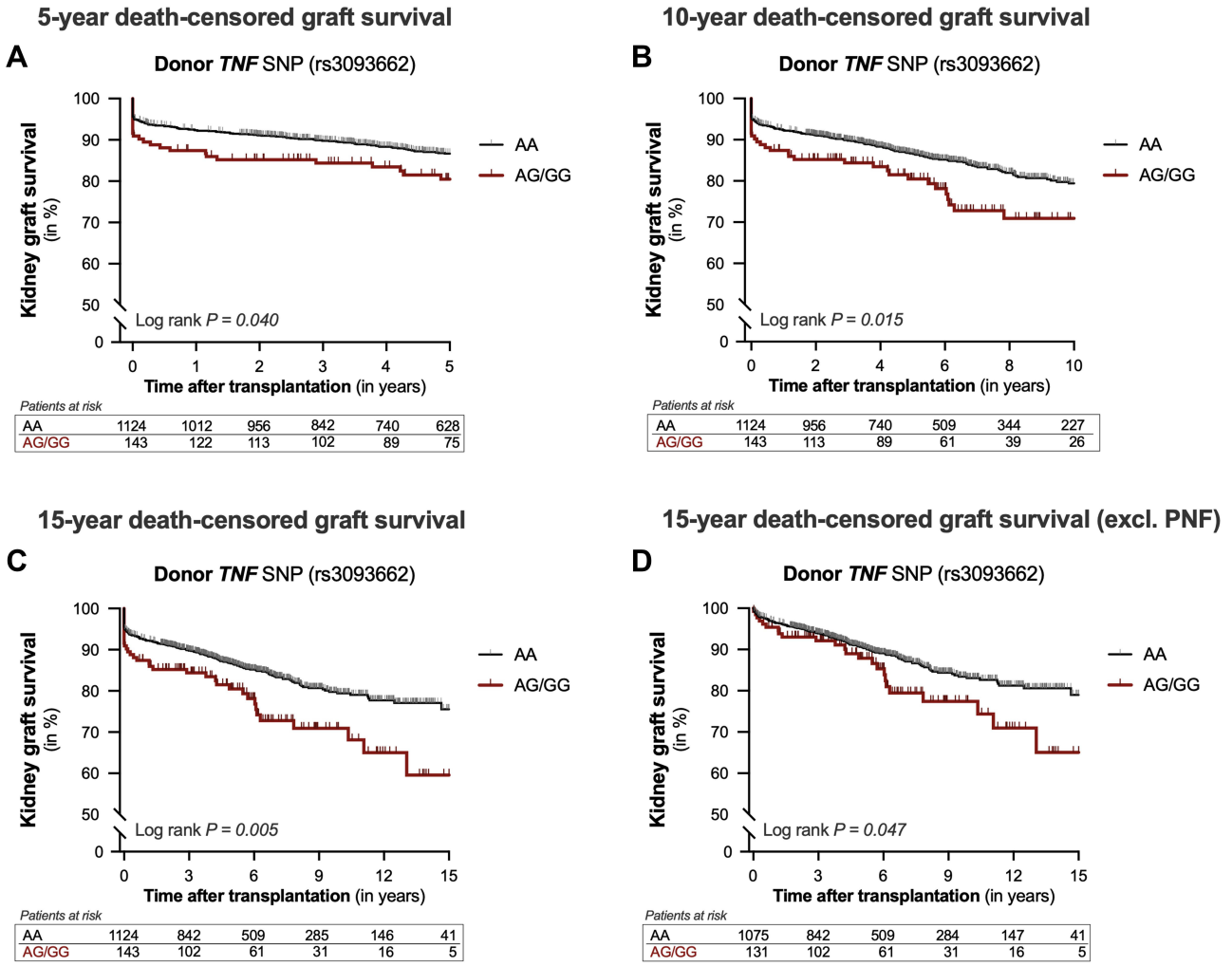

Figure 2 Kaplan–Meier curves for 5-year, 10-year, and 15-year death-censored graft survival after kidney transplantation according to the *TNF* rs3093662 polymorphism in the donor. Cumulative (**A**) 5-year, (**B**) 10-year, and (**C**) 15-year death-censored kidney graft survival according to the presence of the rs3093662 A>G polymorphism in the tumor necrosis factor-alpha gene (*TNF*) in the donor. Furthermore, Kaplan–Meier curves for 15-year death-censored graft survival after kidney transplantation were reanalyzed after excluding donor and recipient kidney transplant pairs with primary non-function (PNF) (**D**). The incidence of graft loss among the groups was compared using the Log rank test.

immune cell functions, (ii) Inhibiting inflammatory mediators, (iii) Protecting kidney cells from apoptosis, and (iv) Stimulating tissue regeneration.[7,10] Application of TNF-α antagonists in kidney transplantation would be especially interesting given the extensive clinical experience with this biological. Recently, a meta-analysis found no significant increase in the infection rate among transplantation patients already on TNF-α inhibitors due to inflammatory bowel disease.[30] However, an observational study, comparing seven renal transplant recipients who resumed their TNF-α inhibitors after transplantation to seven recipients who did not, found that malignancies occurred more frequently.[31] Etanercept, a fusion protein TNF-α inhibitor, has already been tested in kidney transplantation during hypothermic machine perfusion. However, no differences were seen in renal transplant outcomes between the groups.[32] Finally, a Phase 2, double-blind, multicenter, randomized controlled trial of 300 deceased donor kidney transplant recipients has just been completed (NCT02495077). In this trial, a mAb targeting TNF-α (Infliximab) was given to recipients prior to transplantation, in addition to current immunosuppressive regimens, with kidney function at 2 years post-transplantation as the primary outcome. The results of this trial are anxiously awaited by the transplant community and will help define the clinical role of TNF-α inhibitors in kidney transplantation.

The present study found that a *TNF* genetic variant (rs3093662 A>G) in the donor, but not in the recipient, associated with the risk of graft loss after kidney transplantation. Our findings indicate that it is not circulating TNF-α from the

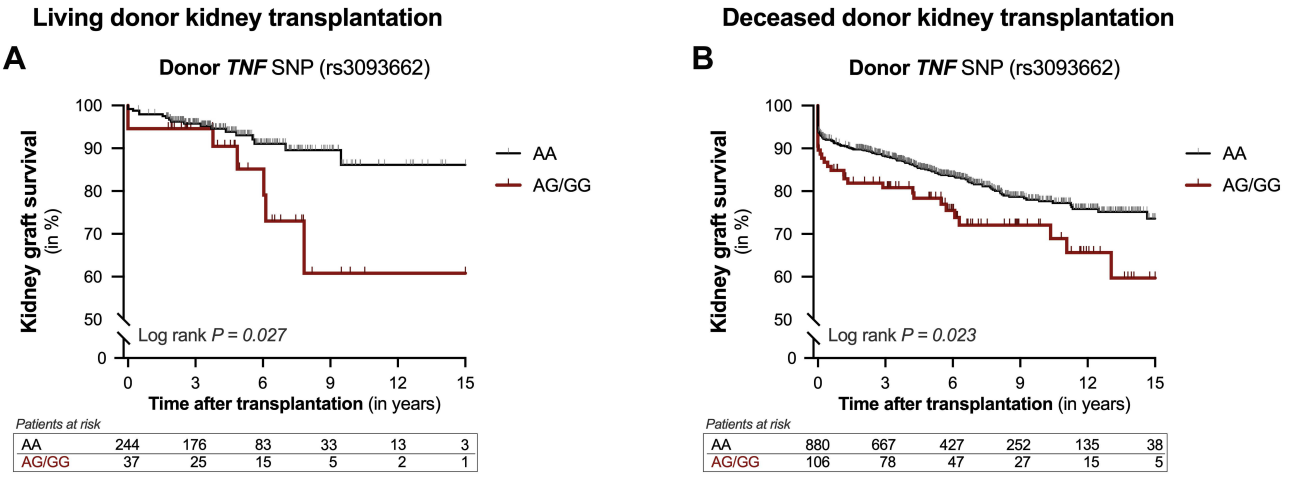

Figure 3 Kaplan–Meier curves for 15-year death-censored graft survival after kidney transplantation according to the *TNF* rs3093662 polymorphism in living and deceased kidney donors. A subgroup analysis for donor type was performed to look at the cumulative 15-year death-censored kidney graft survival according to the presence of the rs3093662 A>G polymorphism in the tumor necrosis factor-alpha gene (*TNF*) in (**A**) living kidney donors and (**B**) deceased kidney donors. The Log rank test was used to compare the graft loss incidence between the different group.

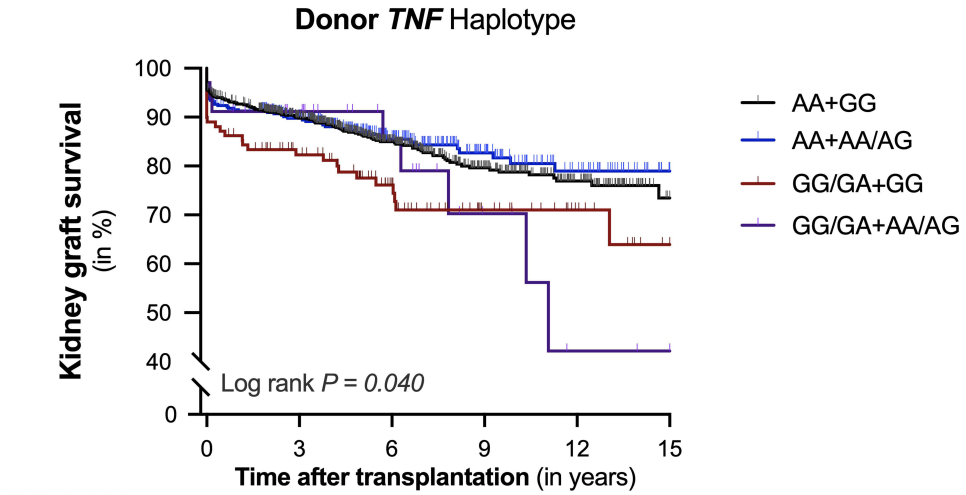

Figure 4 Kaplan–Meier curves for 15-year death-censored graft survival after kidney transplantation according to the *TNF* according to the *TNF* haplotypes in the donor. Cumulative 15-year death-censored kidney graft survival according to the presence of the rs1800629 G>A polymorphism as well as the rs3093662 A>G polymorphism in the tumor necrosis factor-alpha gene (*TNF*) of the donor. Pairs were divided into four groups according to the absence of both (black line), the presence of only the A-allele of the rs1800629 polymorphism (blue line), the presence of only the G-allele of the rs3093662 polymorphism (red line), or the presence of both minor alleles (purple line). Log rank test was used to compare the incidence of graft loss between the groups.

recipient, but rather the release of TNF-α by the allograft that contributes to kidney transplant failure. These results provide important considerations for anti-TNF-α therapies in kidney transplantation. TNF-α inhibitors that are currently used in clinical practice are either antibodies or soluble TNF-α receptors with distinct pharmacodynamic profiles and side-effects related to systemic exposure. However, if the donor kidney is the site of action, small interfering RNA (siRNA) or antisense oligonucleotides could be a more favorable approach due to better drug penetration into the donor kidney.[33] The association we found between donor genetics and transplant outcomes suggests that donor pre-treatment with TNF-α inhibitors should also be considered. Interventions within the donor may protect against a proinflammatory

Table 4 Competitive Analysis of the Associations of Characteristics with Late Graft Loss After Kidney Transplantation

Variables Not in the Equation		Variables in the Equation		
Variables	**P-value**	**Variables**	**P-value**	**Hazard Ratio**
Donor blood type (*AB versus other*)	0.93	rs3093662-G in the donor (A *versus G*)	0.028	1.51 (1.05–2.19)
Donor type (*living versus deceased*)	0.11	Donor age *(in years)*	0.002	1.02 (1.01–1.03)
Cold ischemia time (*in hours*)	0.11	Recipient age *(in years)*	<0.001	0.98 (0.97–0.99)
Warm ischemia time (*in minutes*)	0.08	Recipient blood type (*AB versus other*)	0.001	
Corticosteroids	0.08	Delayed graft function *(yes versus no)*	<0.001	4.00 (3.03–5.30)
Cyclosporin	0.27			

Notes: Multivariable cox regression was performed for 15-year death-censored kidney graft survival with a stepwise forward selection. All variables with a *P*-value < 0.05 in the univariate analysis for late graft loss were included. Data are presented as hazard ratio with 95% confidence interval (CI) and *P*-value. In the final model, the *TNF* rs1800629 G>A polymorphism in the donor, recipient and donor age, recipient blood type and delayed graft function (DGF) were included, whereas donor blood type, donor type, cold ischemia time, warm ischemia time, and use of cyclosporin and corticosteroids were not.

reaction after transplantation, and therefore, donor pre-treatment could be a promising strategy to increase graft function and survival.[34,35] Conversely, donor pre-treatment is not possible until it is known whether other donor organs (ie, the liver, heart, and lungs) also benefit from targeting TNF-α.

The current study has several limitations that warrant consideration. First, a causality of the observed association cannot be established by this observational study as further work must be done to confirm our results. Second, we only investigated the association of two *TNF* polymorphisms with outcome and did not look at *TNF* haplotypes. Third, because of the lack of plasma samples, we could not examine the differences in TNF-α plasma levels between the *TNF* genotypes. However, important strengths of our study include the prospective design, the large sample size, the long follow-up time, and the clinically meaningful endpoint (ie, graft loss).

In conclusion, we found that kidney allografts possessing a *TNF* polymorphism, that has been associated with higher TNF-α levels, have a greater risk of immediate and late graft loss after kidney transplantation. Our study adds to the growing body of literature showing that TNF-α blockade in kidney transplantation has the potential to improve transplant outcomes. Following up on this work with clinical trials using TNF-α inhibitors is necessary to determine the ideal setting, including the timing of intervention, in which these biologicals might be effective.

Abbreviations

DGF, delayed graft function; HLA, human leukocyte antigen; HR, hazard ratio; OD, odds ratio; PNF, primary non-function; mAb, monoclonal antibody; siRNA, small interfering RNA; SNP, single-nucleotide polymorphism; TNF-α, tumor necrosis factor alpha; *TNF*, tumor necrosis factor alpha gene.

Data Sharing Statement

All data generated or analyzed during this study are included in this article and its Supplementary Material. Further enquiries can be directed to the corresponding author.

Statement of Ethics

The current work is in line with the declaration of Helsinki and all subjects provided written informed consent. Furthermore, all kidneys were donated voluntarily with written informed consent, and that this was conducted in

accordance with the Declaration of Istanbul. In addition, this study was approved by the medical ethics committee of the University Medical Center Groningen under file n° METc 2014/077.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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