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Running head: SNPs and kidney allograft rejection

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Abstract

Background: Persistent inflammation and fibrosis have been related to active progression of renal deterioration and reduced survival of kidney transplant. The aim of this study was to determine the impact of single-nucleotide polymorphisms (SNPs) located in regions related to inflammatory and immune processes on the development of chronic renal allograft dysfunction (CRAD).

Methods: A retrospective study was carried out on 276 patients who received kidney transplant (KT). SNPs were genotyped via the SNPlex platform. Statistical analysis was performed with SNPstat and regression logistic analyses were adjusted by age and gender of recipients and donors, cold ischemia time and the number of human leukocyte antigen (HLA) mismatches.

Results: From 276 patients with KT, 118 were non-CRAD and 158 were CRAD. Three SNPs showed significant associations with CRAD development: rs1800471 in *transforming growth factor beta 1 (TGFB1)*, rs5186 in *angiotensin II receptor type 1* (*AGTR1*)**,** and rs699947 in *vascular endothelial growth factor A* (*VEGFA*). GC genotype of rs1800471 was associated with increased odds of CRAD compared to GG genotype (OR= 2.65 (95% confidence interval (CI)= 1.09; 6.47), p= 0.025), as well as AC and AA genotype of rs699947 assuming a dominant model (OR= 1.80 (95%CI= 1.02; 3.20), p= 0.044). Besides, AC and CC genotypes of rs5186 were associated with reduced odds of CRAD assuming a dominant model (OR= 0.56 (95%CI= 0.33; 0.96), p= 0.033).

Conclusion: Our findings suggest that three genes related to immunity and inflammation (rs1800471, rs5186 and rs699947) are associated to susceptibility or protection to CRAD, and might have diagnostic utility in predicting the likelihood of developing CRAD.

Key words: kidney transplant; allograft rejection; SNPs; grow factors; biomarkers

SUBDIVISION - NUMBERED SECTIONS

1. INTRODUCTION

Chronic kidney disease and end-stage renal disease (ESRD) are global public health problems with important social and economic impact, due to their high prevalence and substantial effect on morbidity and mortality [1]. Renal replacement therapy for ESRD includes hemodialysis, peritoneal dialysis, and kidney transplant (KT), where KT is the best therapeutic alternative for most causes of chronic renal failure [1, 2]. Currently, strategies to maintain transplant function and to improve long-term graft survival are important goals in renal transplantation in order to prevent chronic renal allograft dysfunction (CRAD) [3], which is defined as functional and morphologic deterioration of a renal allograft at least 3-6 months after transplantation.

There are several factors that influence graft survival such as the age of recipient and donor, degree of human leukocyte antigen (HLA) matching, cold ischemia time and delayed renal graft function, type of immunosuppression, incidence of acute rejection and time on dialysis before transplantation among others [4]. Persistent inflammation and fibrosis have also been related to active progression of renal deterioration and reduced KT survival [5], therefore both factors provide important diagnostic and therapeutic information for patient management [6]. Besides, KT outcomes may also be influenced by the production of immune mediators, which display genetic inter-individual differences. For example, single-nucleotide polymorphisms (SNPs) located on cytokines may have a crucial impact on graft survival by affecting gene expression [7]. Indeed, it has been proved that changes in pro-inflammatory cytokines levels lead to increase interstitial fibrosis and tubular atrophy and therefore, the risk of allograft rejection [8].

To date, the influence of many inflammatory markers on the KT outcome is not well understood [7, 9]. For this reason, in the present study we have try to determine the impact of SNPs, located in regions related to inflammatory and immune processes, on CRAD development in patients with KT.

2. PATIENTS AND METHODS

2.1. Study design and patients

A retrospective observational study was carried out among patients with ESRD who received KT in the “Hospital Clínico Universitario” from Valladolid (Spain), between December 1995 and October 2008. The total number of kidney transplants performed in this period was 440. Only those patients who gave their written consent were included in the study, being approved by the Institutional Ethics Committee. Demographic and clinical data were obtained from medical records.

Patients who were younger than 18 years, those that had unavailable DNA sample and/or that unsigned informed consent, were ruled out. In total, 276 patients were included and divided into 2 groups of study according to the kidney graft outcomes: a) Non-CRAD: patients with stable transplant or absence of CRAD; b) CRAD: patients that developed CRAD. We have considered as CRAD any decrease ≥30% of the inverse of creatinine (1/Cr) after the third month of transplantation, using as baseline the highest value of 1/Cr in the first three months after transplantation [10]. In our hospital, biopsy is not performed to monitor the evolution of renal transplantation per protocol.

We also included 288 normal subjects (control-group) from the same hospital. These subjects were routinely discharged from the general medicine service with an age and gender distribution similar to the case-group patients. In order to ensure homogeneity, all patients were Caucasian.

Regarding immunosuppression protocol, an anti-interleukin-2 receptor monoclonal antibody (basiliximab) and steroids were used as induction treatment. For maintenance immunosuppression, cyclosporine and steroids were used until 2002; tacrolimus and steroids after 2002. In addition to this, mycophenolate mofetil was also applied when transplant came from an elderly donor, it was a second transplant and/or patients developed acute tubular necrosis.

2.2. SNPs selection

Published SNPs at Pubmed database (http://www.ncbi.nlm.nih.gov/pubmed/) located in genes involved in inflammatory and immune pathways, as well as those related to kidney transplantation toleration or associated processes, were selected. SNPs located at regulatory regions were prioritized. Furthermore, several databases were reviewed: a) SNPs: HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) and dbSNP databases (http://www.ncbi.nlm.nih.gov/SNP/); b) Pathways: GeneOntology (http://www.geneontology.org/) and KEGG (http://www.genome.jp/kegg/pathway.html) for pathways analysis.

Moreover, for each significant SNP, the biological implications were analyzed "in silico" via web-tools: SMART (http://smart.embl-heidelberg.de/) for identifying the protein domains, PolyPhen (http://genetics.bwh.harvard.edu/pph/) for predicting possible impact of an amino acid substitution on the structure and function of a protein, PATROCLES (http://www.patrocles.org/) for identifying putative microRNA binding sites, ESEfinder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home) to identify exonic splicing enhancers binding sites and TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) for searching transcription factor binding sites.

2.3. DNA samples and genotyping

DNA was extracted from whole blood by the Chemagic Magnetic Separador Module1, CHEMAGEN® which uses a magnetic particles system to obtain DNA. The quantity of recovered DNA was quantified by using PicoGreen® dsDNA Quantitation Reagent (Molecular Probes, Inc., Eugene, Oregon, USA).

All SNPs were genotyped at the Spanish National Genotyping Centre (CeGen; http://www.cegen.org/) by the SNPlex genotyping system 48-plex (Applied Biosystems, Foster City, CA, USA) following the manufacturer's recommended protocol (http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_042019.pdf). As quality control, two Centre d'Etude du Polymorphisme Humain (CEPH) samples (NA10860 and NA10861) from the HapMap database were included in all genotyping assays [11].

2.4. Statistical Analysis

All SNPs from the control group were analyzed for Hardy-Weinberg equilibrium (HWE) by using the Pearson Chi-square ($\chi 2$) statistic. Only SNPs that fulfilled HWE were included in the association analysis.

In order to perform the association analysis, a logistic regression model was carried out by using SNPstat software (http://bioinfo.iconcologia.net/SNPStats_web) [12]. We have considered the most influential clinical factors as adjusted variables (age and gender of recipients and donors, cold ischemia time and the number of HLA mismatches), in order to correct the association results. Five inheritance models were tested (co-dominant, dominant, recessive, over-dominant and additive). For each SNP, likelihood ratio test (LRT), and Akaike's information criteria (AIC) were used to select the inheritance model that best fits the data. The equation that defines the logistic model is: $\log (p/1-p)= \alpha+\beta G+\gamma Z$; p being the probability, *G* the categorical variable with the polymorphisms codified, *Z* the variables to adjust the model (α, β

and γ must be estimated). Odds ratios (OR) and 95% confidence intervals (CI) were calculated to test the relative risk for association.
Besides, multiple testing corrections were performed by SFDR (Stratified False Discovery Rate) software version 1.6. (http://www.utstat.utoronto.ca/sun/Software/SFDR/index.html). Significance was fixed at p value < 0.05.

3. RESULTS

3.1. Patients

Table 1 shows the clinical characteristics of all patients included in our study: 118 were in the Non-CRAD group, and 158 in the CRAD. Chronic glomerulonephritis was the most frequent cause of ESRD in both groups. The most frequent causes of death of donors was cerebrovascular accident followed by traumatic brain injury. Finally, CRAD groups had higher cold ischemia time than non-CRAD group (p<0.05).
The mean follow up of patients after transplantation was 5.32±3.02 years. The percentage of positive panel-reactive antibodies (PRA) was 10% in no-CRAD versus 13% in CRAD group. Induction treatment was used in 86% no-CRAD patients versus 71.2% CRAD (p>0.05).
Table 1. Clinical characteristics of patients with renal allograft transplant.

Characteristics	Non-CRAD	CRAD
No.	118	158
Receptor		
Age †	50.5 (15.2)	51.5 (18)
Male *	54 (45.8)	54 (34.2)
Primary disease*		
Glomerulonephritis	34 (28.8)	48 (30.4)
Arterial hypertension	14 (11.9)	16 (10.1)
Diabetic nephropathy	9 (7.6)	11 (7.0)
Tubulointerstitial nephritis	10 (8.5)	18 (11.4)
Obstructive uropathy	6 (5.1)	5 (3.2)
Vascular causes	4 (3.4)	1 (0.6)
Polycystic kidney disease	18 (15.2)	19 (12.0)
Others	23 (19.5)	40 (25.3)
Donor		
Age †	45.0 (27.0)	53 (21)
Male *	81 (68.6)	105 (66.5)
Cause of death*		
CVA	56 (47.5)	89 (56.3)
TBI	51 (43.2)	55 (34.8)
Others	11 (9.3)	14 (8.9)
Transplant factors		
HLA A mismatches*		
0	68 (57.6)	81 (51.3)
1	46 (39.0)	73 (46.2)
2	4 (3.4)	4 (2.5)
HLA B mismatches*		
0	79 (66.9)	110 (69.6)
1	37 (31.4)	46 (29.1)

2	2 (1.7)	2 (1.3)
HLA DR mismatches*		
0	13 (11.0)	16 (10.3)
1	94 (79.7)	131 (82.9)
2	11 (9.3)	11 (7.0)
Cold ischemia time (minutes) †	693.5 (309.2)	846 (420)

CRAD: chronic renal allograft dysfunction; NCRAD: no chronic renal allograft dysfunction; CVA: cerebrovascular accident; TBI: traumatic brain injury; HLA, human leukocyte antigen.
*Absolute number (percentage). †Median (interquartile rank). NA: not applicable

3.2. SNPs analysis

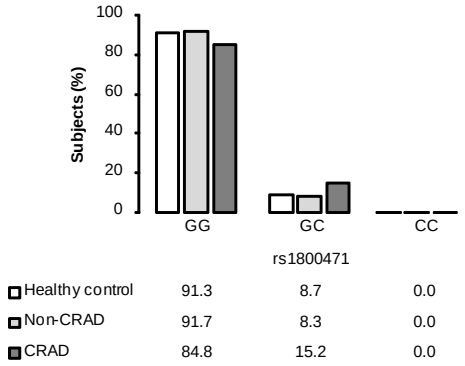
Although 48 SNPs were selected and genotyped, analyses were only performed on 34 (see **Supplemental Digital Content 1**). Fourteen SNPs had to be discarded due to different reason: a) 6 SNPs display genotyping problems (rs1570360, rs1808593, rs2070744, rs4762, rs5498, rs55634318); b) 2 SNP were monomorphic (rs175176 and rs470206); and c) 6 SNPs exceed 10% of missing values (rs4311, rs699) and/or had a minimum allele frequency (MAF) less than 0.05 (rs1800825, rs2071231, rs4986790, rs5743708).

HWE was fulfilled for all SNPs in the control group (288 control subjects).

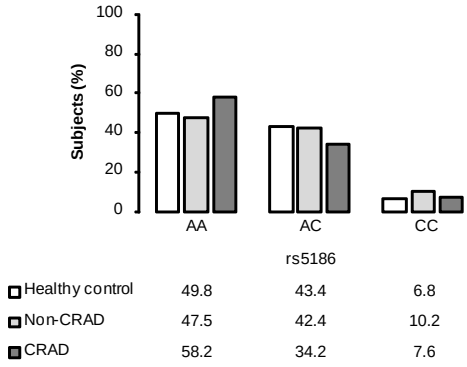
3.3. Association analysis with CRAD

From clinical point of view, non-CRAD group seems to show better baseline characteristics, which could interfere with the analysed outcome. In order to avoid such interferences, logistic regression analysis was performed by adjusting for age and gender of recipients and donors, as well as cold ischemia time and HLA mismatches. Thus, we found that three SNPs were significantly associated to CRAD. Genotypic frequencies are shown in **Figure 1.**

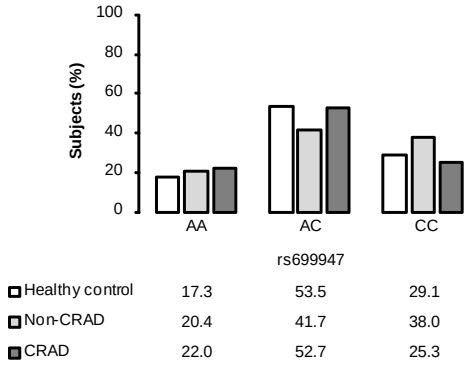
Figure 1. Genotypic frequencies of SNPs associated with chronic renal allograft dysfunction (CRAD). Abbreviations: CRAD, chronic renal allograft dysfunction; Non-CRAD, non chronic renal allograft dysfunction; rs1800471, SNP in *transforming growth factor beta 1 (TGFB1);* rs5186, SNP in *angiotensin II receptor type 1* (*AGTR1*); rs699947, SNP in *vascular endothelial growth factor A* (*VEGFA*).



	GG	GC	CC
Healthy control	91.3	8.7	0.0
Non-CRAD	91.7	8.3	0.0
CRAD	84.8	15.2	0.0



	AA	AC	CC
Healthy control	49.8	43.4	6.8
Non-CRAD	47.5	42.4	10.2
CRAD	58.2	34.2	7.6



	AA	AC	CC
Healthy control	17.3	53.5	29.1
Non-CRAD	20.4	41.7	38.0
CRAD	22.0	52.7	25.3

The results of association analysis were as follows (**Table 2**): On the one hand, GC genotype of rs1800471 located at *transforming growth factor beta 1 (TGFB1)* gene as well as CA-AA genotype of rs699947 at *vascular endothelial growth factor A* (*VEGFA*) gene, were associated with increased odds of CRAD assuming a dominant model (OR= 2.65, p= 0.025; OR= 1.80, p= 0.044, respectively). On the other hand, AC and CC genotypes of rs5186 located at *angiotensin II receptor type 1* (*AGTR1*) gene, were associated to reduced odds of CRAD assuming a dominant model (OR= 0.56, p= 0.033) (**Table 2**).

Table 2. SNPs associated with chronic renal allograft dysfunction (CRAD).

SNP	Gene	Inheritance model	Genotype	Non-CRAD	CRAD	OR (95% CI) (*)	p-value
rs1800471	*TGFB1*	---	GG	100 (91.7%)	127 (84.7%)	1.00	
			GC	9 (8.3%)	23 (15.3%)	2.65 (1.09-6.47)	0.025
rs5186	*AGTR1*	Dominant	AA	56 (47.5%)	91 (58.3%)	1.00	
			AC-CC	62 (52.5%)	65 (41.7%)	0.56 (0.33-0.96)	0.033
rs699947	*VEGFA*	Dominant	CC	41 (38%)	38 (25.7%)	1.00	
			CA-AA	67 (62%)	110 (74.3%)	1.80 (1.02-3.20)	0.044

Abbreviations: CRAD, chronic renal allograft dysfunction; Non-CRAD, non chronic renal allograft dysfunction; *TGFB1, transforming growth factor beta* 1gene*; AGTR1*, *angiotensin II receptor type 1* gene; *VEGFA, vascular endothelial growth factor A* gene; OR, odds ratio; 95% CI, 95% of confidence interval; p-value, level of significance.

(*), OR and "p-value" were adjusted by in a adjusted by age and gender of receptor and donor, ischemia time, and HLA mismatches.

4. DISCUSSION

The finding of genetic markers corresponding to a predisposition for CRAD could help to predict the risk of developing rejection and also to improve the understanding of the pathways involved in the disease pathogenesis. In our study, three SNPs located in genes related to immune and inflammatory processes were significantly associated to CRAD (rs1800471 and rs699947 with susceptibility; and rs5186 with a protective effect).

The missense rs1800471 (C/G) is located on chromosome 19, at exon 1 of *TGFB1* gene and it produces a p.Arg25Pro change. This gene encodes for TGFB1 which is a multifunctional cytokine involved in the proliferation of fibroblasts and it can also inhibit the atheromatous process [13]. Rs1800471 could play a key role in the TGFB1 function because it is located in a signal peptide region of *TGFB1* (SMART) and therefore, it is probably involved in the protein secretion and the addressing. When we analysed the effect of the C allele, four exonic splicing enhancers (ESE) binding sites responsive to human splicing regulatory proteins (SF2/ASF and SC35) were identified. However, only one ESE binding sites responsive to SF2/ASF remain when G allele was present (ESEFinder). Therefore, rs1800471 might have an important regulatory effect on alternative splicing, which is a major contributor to both proteomic diversity and control of gene expression levels. Splicing disruption can lead to a wide range of human diseases [14] and in this case, could have an important implication on renal pathology. On the other hand, this SNP produces an amino acid change, but it has no any effect on the protein structure (Polyphen). We have also found that conflicting results have been published regarding the effect of this SNP on CRAD. Some studies have detected no significant associations with transplantation rejection [15] and others have described an association between GG genotype and CRAD development [8]. In our study we have observed that GC genotype was the responsible of CRAD development. These data are also consistent with other reported articles, where it have been described that C and GC carriers are more likely associated to diseases strongly linked to renal failure such as cardiovascular disease [16-18] and diabetic nephropathy [19]. In any case, further studies with larger sample size are needed to verify the association of rs1800471 with CRAD.

On the other hand, rs699947 (C/A) is located on chromosome 6, at the promoter region of *VEGFA* gene, which encoded for a protein involved in angiogenic processes. VEGFA plays a crucial role in the kidney physiology, being involved in the filtration, integrity and permeability of the glomerular basement membrane [20]. We analyzed *in silico* the effect of rs699947 alleles and we found that C allele generates a transcription factor binding site for GATA-1 and GATA-2, whereas A allele disrupt this target site (TFSearch). For this reason, rs699947 could regulate its own expression and, to influence VEGFA production and secretion. Another hypothesis related to a reduced expression of VEGFA when A allele is present, could be based on the fact that it generates a target site for hsa-miR-1202 (CTGGCA**A**) (PATROCLES database). MicroRNAs are able to silence gene expression by mRNA degradation or blocking the mRNA translation [21]. These findings support that different SNP allele can lead to variations in VEGFA expression levels, where A allele carriers might show a reduced VEGFA expression. In fact, it has previously published that serum VEGFA level is lower in patients with CA/AA genotype than those with CC genotype [22]. Therefore, the fact that CA and AA genotypes are associated with CRAD, suggest that an increased VEGFA production could have a protective effect on kidney allograft outcomes. Besides, previous reports have also described an association between high production of VEGFA and better survival of both peritoneal dialysis patients and kidney graft recipients compared to low production of VEGFA [23, 24].

Significant association was also found for rs5186 (A/C), a polymorphism located on chromosome 3, at 3'UTR region of *AGTR1* gene. This gene encodes for a protein that

modulates the renin-angiotensin system and it is involved in the preservation of renal hemodynamic environment by raising systemic blood pressure [25]. Due to its location at the 3´UTR, this polymorphism could affect the posttranscriptional regulation [26]. In fact, it has been previously published that the A allele generates a target site for hsa-miR-155 (AGC**A**TTA) (PATROCLES) which could decrease the *AGTR1* expression.[27]. Our findings suggest that AA genotype and therefore, reduced AGTR1 levels, may be associated with susceptibility effect to CRAD. Although there is little information about the relationship of this SNP and CRAD, our results are consistent with other articles where AA genotype has been associated with increased risk of kidney deterioration [28, 29] and renal deterioration-related diseases such as cardiovascular disease [5].

The arguments mentioned above may indicate that patients with specific genotypes of rs1800471 (GC), rs699947 (CA-AA) and/or rs5186 (AA) that correspond to reduced levels of TGFB1, VEGFA and AGTR1, respectively, could show an increased risk of CRAD. Supporting our findings, it has been published that some of the angiotensin II effects mediated through its receptor, AGTR1, are carried out via mechanisms that involve TGFB1 and VEGFA signalling pathways [30]. Therefore, these pathways seem to be interrelated in the renal physiopathology, providing higher coherence and consistency to our results.

Finally, our study present some limitations that must be taken into account for a correct interpretation of our data. This is a retrospective case-control study with a low number of patients that might introduce some uncontrolled bias. Regarding the signification, when applying the false discovery rate (FDR) correction for multiple tests, adjusted “p-values” for each hypothesis were not significant. However, there is a considerable controversy about adjusting the “p-value” after multiple tests on clinical-orientated studies [10, 31]. It is important to have in mind that in our study there is a hypothesis supported by theory and previous reports in patients with renal disease (**see Supplemental Digital Content 1**), therefore we are not doing a random search of a meaningful result, and our results should not be affected by the fact of carrying out a high number of statistical tests. Moreover, since p-value is depending on the sample size, it may be possible that we did not find any significant adjusted p-value due to such a size-limited population. Thus only big effects would be detected in small populations. In addition to this, it has to be taken into account that the effect size of our study is low due to the fact that, complex human diseases are under the control of many genes that contribute each of them with modest individual effects [32].

In conclusion, our findings suggest that 3 genes related to immunity and inflammation (rs1800471, rs5186 and rs699947) are associated to susceptibility or protection to CRAD, and might have diagnostic utility in predicting the likelihood of developing CRAD.

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SUPPLEMENTAL MATERIAL

Table SDC 1. Description of gene polymorphisms included in the study**.**

Gene	SNP ID	Change	Chr	Disease Association
Angiotensin I converting enzyme (ACE)	rs12449782	A/G	17	Nephropathy [1; 2] Cardiovascular diseases [3]
	rs1800764	C/T	17	Nephropathy [1; 2; 4]
Angiotensin II receptor, type 1 (AGTR1)	rs5186	A/C	3	Chronic kidney failure [5]
Chemokine (C-C motif) ligand 5 (CCL5)	rs2107538	C/T	17	Type 1 diabetes [6; 7] Chronic graft-versus-host disease [8]
Chemokine (C-C motif) ligand 2 (CCL2)	rs4586	C/T	17	Cardiovascular disease [9] Inflammation and infection [10]
Intercellular adhesion molecule 1 (ICAM1)	rs1799969	A/G	19	Cardiovascular diseases [19]
Interleukin 1, beta (IL1B)	rs1143634	C/T	2	Cardiovascular diseases [11] Obesity and metabolic syndrome [12] Type 2 diabetes [13]
Interleukin 1 receptor antagonist (IL1RN)	rs419598	C/T	2	Cardiovascular disease [14] Inflammation [15]
	rs2234676	A/G	2	Inflammation and allergy [16]
Interleukin 4 (IL4)	rs2070874	C/T	5	Inflammation and autoimmune disorders [17]
	rs2243248	G/T	5	Inflammation and autoimmune disorders [18]
Interleukin 4 receptor (IL4R)	rs1801275	A/G	16	Inflammation and autoimmune disorders [19; 20]
Interleukin 6 (IL6)	rs1800795	C/G	7	Chronic kidney failure [21] Nephropathy [22] Type 2 diabetes [23] Cardiovascular diseases [23; 24; 25]
	rs1800796	C/G	7	Chronic kidney failure [21] Type 2 diabetes and obesity [21] Nephropathy [26; 27] Cardiovascular disease [11; 25]
	rs1800797	A/G	7	Cardiovascular disease [25] Metabolic syndrome [28]
Interleukin 10 (IL10)	rs1800871	C/T	1	Type 2 diabetes [40] Cardiovascular disease [41] Chronic kidney failure[42]
	rs1800872	A/C	1	Type 2 diabetes [29] Cardiovascular disease [11]
	rs1800896	A/G	1	Type 2 diabetes [29] Cardiovascular disease [30]

Interferon, gamma (IFNG)	rs2430561	A/T	12	Kidney transplant failure [31] Nephropathy [32] Type 1 diabetes [33] Autoimmune disorders [34]
Matrix metallopeptidase 1 (MMP1)	rs1799750	-/G	11	Chronic kidney failure [35] Cardiovascular disease[36]
Matrix metallopeptidase 2 (MMP2)	rs243865	C/T	16	Cardiovascular disease [37] Inflammation [38]
Intergenic region - STAT4 binding site	rs301640	A/G	13	Inflammation [39]
Nitric oxide synthase 3 (NOS3)	rs3918226	C/T	7	Inflammation [40] Cardiovascular diseases [41]
	rs7830	G/T	7	Cardiovascular diseases [41]
Hepatitis A virus cellular receptor 1 (HAVCR1)	rs41297579	A/G	5	Inflammation and infection [42]
Hepatitis A virus cellular receptor 2 (HAVCR2)	rs1036199	A/C	5	Type 1 diabetes [43]
	rs10515746	A/C	5	Type 1 diabetes [43]
TIMP metallopeptidase inhibitor 3 (TIMP3)	rs5749511	C/T	22	Cardiovascular disease [44]
Toll-like receptor 2 (TLR2)	rs4696480	A/T	4	Inflammation [45]
Toll-like receptor 4 (TLR4)	rs4986791	C/T	9	Type 2 diabetes [46]
Transforming growth factor, beta 1 (TGFB1)	rs1800471	C/G	19	Nephropathy [68] Cardiovascular diseases [70]
Tumor necrosis factor (TNF)	rs1800629	A/G	6	Kidney transplant failure [47; 48] Type 1 diabetes [49] Inflammation and autoimmune disorders [50; 51] Metabolic syndrome [28]
Vascular endothelial growth factor A (VEGFA)	rs699947	A/C	6	Cardiovascular disease [52] Chronic graft-versus-host disease [53]
	rs833061	C/T	6	Chronic graft-versus-host disease [53]

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