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Original Article

Interleukin 17F Gene Polymorphisms and Chronic Kidney Allograft Failure

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Abstract

Background and objectives: Polymorphisms of the interleukin (*IL*)-17 proinflammatory cytokine family (*IL17A* and *IL17F*) have been associated with kidney chronic allograft failure (CAF). To date, the impact of heritable differences in *IL17F* genes and CAF among kidney transplant patients from North America has not been reported. The objective of the study was to assess the association of five distinct polymorphisms in the *IL17F* gene with histopathological changes in chronic kidney allograft failure.

Methods: Two hundred eighteen kidney transplant recipients were enrolled. Surveillance biopsies were performed to evaluate 11 distinct histological markers and the combined grade of interstitial fibrosis and tubular atrophy, 6 to 12 months post-transplant. Using direct sequencing, the *IL17F* polymorphisms (*-1507C/T rs1889570*, *-1165A/G rs1266828*, *-5046C/T rs7771511*, *-6328G/A rs766748*, and *-7488A/G rs763780*) were genotyped in the 10 healthy volunteer samples followed by all kidney transplant recipients were genotyped for five *IL17F* gene polymorphisms using polymerase chain reaction and sequence-specific primers. The association was evaluated using both univariate and multivariate logistic regression analysis.

Results: We observed weak associations of TC genotype of *IL17F*-1165 (*rs1266828*) and allele of *IL17F* -1507C (*rs1889570*) with glomerular sclerosis and interstitial fibrosis and tubular atrophy ($p = 0.017$ and $p = 0.03$) respectively. Allele C of *IL-17* -1165C/T (*rs1266828*) was associated with better glomerular sclerosis ($p = 0.004$, odds ratio = 0.39) score.

Conclusions: Our findings demonstrate that *IL17F* SNPs were not associated with CAF and support our prior published results that production of pro-inflammatory cytokines is not a strong predictor of CAF.

Introduction

Chronic allograft failure (CAF) is characterized by typical histological changes that include arteriosclerosis, interstitial fibrosis, and tubular atrophy.[1] Previous evidence indicates that T cells are the main population among the immune effectors that infiltrate a chronically rejected allograft.[2,3] There are various subsets of CD4+ T cells, including the classical Th1 and Th2 cells, Th17 cells, follicular helper T cells, and regulatory T cells. Multiple evidence suggests a clear association between Th17 and acute allograft rejection.[2,3]

Owing to the genetic diversity of the host, the immune system's ability to respond to alloantigens contributes to several events that together lead to tubular damage, interstitial fibrosis, and lead to graft failure. Cytokines are the chief mediators of inflammation and play an important role as regulators of inflammation. Polymorphisms located in the regulatory or coding region of cytokine genes influence transcription and production of cytokines either as a low or high production.[4–6] We have previously shown that low interleukin (*IL*) 10 producing genotypes have a high correlation

Keywords: Chronic kidney allograft failure; *IL17*; Polymorphism; PCR-sequence specific primer; Sequencing.

Abbreviations: BMI, body mass index; CAF, chronic allograft failure; CAD, Cadaver; CI, confidence interval; CMV, cytomegalovirus; HLA, human leukocyte antigen; i, interstitial inflammation; IF/TA, interstitial fibrosis/tubular atrophy; *IL*, interleukin; OR, odds ratio; PCR, polymerase chain reaction; PCR, polymerase chain reaction; SSP, sequence specific primer; SNP, single nucleotide polymorphism; TSB, transplant surveillance biopsy.

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with graft inflammation and histology markers of CAF, however, no association was found with any of the production-affecting polymorphisms in the genes encoding pro-inflammatory cytokines.[7] *IL17* is regarded as a crucial cytokine that bridges the gap between the innate and adaptive immune responses. It is chiefly produced by T cells known as Th17 cells, additionally by other cell types, including CD8+ T cells and γδ T cells. This cytokine promotes inflammation and recruits neutrophils to sites of infection or inflammation.[8] *IL17* is linked to the development of autoimmune diseases such as rheumatoid arthritis, psoriasis, and multiple sclerosis. In these conditions, excessive *IL17* production contributes to chronic inflammation and tissue damage. Human and experimental models have shown an elevated level of *IL17* mRNA and proteins for renal allograft rejection.[9–11]

Indeed, the synthesis and regulation of cytokines like *IL17* have a genetic component that can influence their production and function within the immune system. The genetic background of an individual can contribute to variations in cytokine levels, which in turn may affect susceptibility to various diseases, including autoimmune conditions and transplant rejection. These genetic associations known as single nucleotide polymorphisms (SNPs) can lead to changes in the structure or expression of cytokines, potentially impacting their biological activities and the immune responses they regulate. The *IL17* genes are located at the 6p12 region of chromosome 6. Genetic polymorphisms in the *IL17A* and *IL17F* have been studied extensively in relation to autoimmune diseases and transplantation outcomes which may impact the immune response to the transplanted organ and have been linked with the association of chronic inflammatory diseases such as ulcerative colitis,[12] Bahcet′s disease,[13] asthma,[14] or inflammatory bowel disease.[15,16]

To date, the impact of heritable differences in *IL17* genes and CAF among kidney transplant patients from America and North America has not been reported. Many of the studies have evaluated *IL17A* and *IL17F* gene polymorphisms′ effects on long-term kidney allograft function, however, none of the studies has reported the association of *IL17F* gene polymorphism at the genotype level with delayed graft function and chronic kidney allograft failure. Recently, Romanowski *et al.*,[17] evaluated the impact of the *IL17* gene polymorphisms (*IL17A* and *IL17F*) in 269 Caucasian deceased donor renal transplant recipients on long-term kidney allograft function. They reported that *IL17A* gene promoter polymorphism was associated with significantly decreased long-term kidney transplant function. However, none of the genotypes of *IL17F* was directly associated with the delayed graft function. In the present study, we used various histological scores as clinical endpoints for chronic kidney allograft failure to assess the significance of *IL17F* polymorphisms. The objective of the present study was to examine the association of all known five SNPs of *IL17F* gene polymorphisms (*-1507C/T rs1889570*, *-1165A/G rs1266828*, *-5045C/T rs7771511*, *-6328C/T rs766748* and *-7488G/A rs763780*) with transplant surveillance biopsies (TSBs) in 218 transplanted kidney recipients such as glomerular sclerosis (gs), interstitial fibrosis/tubular atrophy (IF/TA), tubular atrophy (ct), mesangial matrix increase (mm), interstitial fibrosis (ci), fibrous intimal thickening (cv), arteriolar hyaline thickening (ah), interstitial inflammation (i), tubulitis (t), glomerulitis (g), allograft glomerulopathy (cg) and intimal arteritis (v) for chronic kidney allograft failure. The five *IL17* SNPs were selected because more than 90% association have been linked with kidney allograft function in previous studies.[1–3,7,17] We expect that these SNPs may impact post-transplant immunological response against the transplanted kidney. We observed weak associations or no associations between the studied *IL17F* gene polymorphisms with glomerular sclerosis and IF/TA scores. We are in concordance with other studies and one of our previous studies, reported no association.[7] A lack of association between *IL17F* SNPs and CAF following kidney transplantation was observed when more stringent criteria were applied.

Material and methods

Subjects

From a total of 536 renal transplants performed at the Southern Alberta Transplant Program between 1997 and 2006, a cohort of 218 renal patients was evaluated in this retrospective study. The availability of recipients′ DNA and the corresponding 6- to 12-month follow-up of TSB results were two criteria for inclusion. The control group consisted of 10 healthy individuals and was used for optimization purposes. Written informed consent from all the patients was obtained before transplantation for TSBs to be used. Recipient demographic data were collected using the ALTRA database and health record censoring. All procedures performed in studies involving human participants were in accordance with the Helsinki declaration or comparable ethical standards. Ethical clearance (ethics ID 24259) was obtained from University of Calgary, Calgary, Canada, and written informed consent was obtained from all individual participants included in the study.

Histologic quantitation of TSBs

To monitor the function of the graft, kidney transplant patients routinely undergo TSBs of the kidney performed at 6 to 12 months after renal transplants to enable early detection of acute and chronic histologic alterations as well as for subsequent intervention. TSBs were performed using 18-gauge needles under ultrasound guidance to obtain two core samples. Hematoxylin-eosin, Masson′s trichrome, periodic acid-Schiff, and periodic acid-Schiff-methenamine-silver stains were used for developing biopsy slides for the TSBs′ paraffin and plastic sections. A transplant pathologist blinded to the treatment and original case diagnosis, independently reviewed each biopsy under light microscopy. TSBs were assessed according to numerous histologic factors only if the biopsy specimen had at least one artery and seven glomeruli.[18] Enrolled patients were categorized based on histological grades scored in surveillance biopsy. Histological grading was based on the following parameters: glomerular sclerosis (gs), interstitial fibrosis/tubular atrophy (IF/TA), tubular atrophy (ct), mesangial matrix increase (mm), interstitial fibrosis (ci), fibrous intimal thickening (cv), arteriolar hyaline thickening (ah), interstitial inflammation (i), tubulitis (t), glomerulitis (g), allograft glomerulopathy (cg) and intimal arteritis (v). Each parameter has four score categories ranging from 0 to 3 [0 (normal), 1 (mild), 2 (moderate), and 3 (severe)]; a score ≥ 1 was considered high and a score < 1 was considered low.[18]

DNA extraction

The genomic DNA from the 200 μL buffy coat was extracted using the Qiagen Kit (Qiagen QIAamp DNA Blood Mini Kit, Qiagen Inc., Mississauga, ON, Canada), according to the manufacturer's recommendations.

Sanger sequencing

Genomic DNA samples from 10 healthy individuals were geno-

Table 1. Primers used for *IL17* gene sequencing

Primer name	Primer sequence
1507 Sequencing primer forward	5′-CCTTCCTTCCTCCTGGGTAG-3′
1507 Sequencing primer reverse	5′-AACTTCTCCTGCCCACCTTT-3′
1165 Sequencing primer forward	5′-CAGGTCTGCCTGACATCAAA-3′
1165 Sequencing primer reverse	5′-CCCTGGATGGAAGAAATGAA-3′
5667 Sequencing primer forward	5′-GTGTAATTCCAGGGGGAGGT-3′
5667 Sequencing primer reverse	5′-GGCTTGCCTTTCTGAGTGAG-3′
6328 Sequencing primer forward	5′-GCAAAGAGCCAGAAAATTCG-3′
6328 Sequencing primer reverse	5′-CTTGGAAGACCAAGCACTCC-3′
7488 Sequencing primer forward	5′-CCATCCGTGCAGGTCTTATT-3′
7488 Sequencing primer reverse	5′-TGTACAGGCCCAGTGTAGGAA-3′

IL-17, interleukin 17.

typed by direct sequencing for each of the studied polymorphisms. Five SNPs screening in the *IL17F* gene was performed with five pairs of primer sets by polymerase chain reaction (PCR) followed by Sanger sequencing (Table 1) using dideoxynucleotide chain terminators labeled with different fluorescent dyes (BigDye Terminator V1.1 cycle sequencing kit; Applied Biosystems Inc., Waltham, MA, USA). The thermal cycling conditions involved one cycle of 5 m at 95 °C, and then 30 cycles of 30 s at 96 °C, 10 s at 58 °C and 4 m at 72 °C. Fluorochrome-labeled DNA fragments were ethanol purified, heat denatured, and electrophoresed by capillary electrophoresis on an Applied Biosystems 3130 genetic analyzer (Applied Biosystems Inc.). The analysis of sequence was done by genetic analyzer software and representative results are reported in Figure 1.

Genotyping of IL17F polymorphisms using PCR–SSP

PCR-sequence specific priming (SSP) was performed to detect the single nucleotide variations in *IL17F* gene for *-1507C/T* (*rs1889570*), *-1165A/G* (*rs1266828*), *-5045C/T* (*rs7771511*), *-6328C/T* (*rs766748*) and *-7488 C/T*(*rs763780*) using allele-specific primers (Table 2). PCR–SSP was optimized based on the sequencing results to ensure accurate results. The primers were designed with optimized length, %GC content, and Tm. Gene sequencing samples served as positive controls and optimized the conditions for PCR–SSP-based genotyping of *IL17F* variants. SNPs were optimized to a unique reaction condition and composition. The procedure involved the amplification of five fragments and subsequent use of the amplicons for each SNP as a DNA template for sequencing. The thermal cycling involved one step of 2 m at 96 °C, 40 cycles of 96 °C for 15 s with annealing at 58 °C for 30 s and extension of 72 °C for 45 s, and one step of extension for 3 m at 72 °C. Each reaction contained primers specific for nonpolymorphic *HLA-DRA* gene: FDRA360 (5′-GAGGTAACTGTGCTCACGAACAGC-3′) and RDRA595 (5′-GGTCCATACCCCAGTGCTTGAGAAG-3′). The 283 bp band produced by these primers serves as a quality control and works as an internal positive control. The PCR-amplification products were analyzed by ethidium bromide-stained agarose gel (Fig. 1).

Statistical analysis

Fisher's exact test was used to analyze the difference between categorical variables. A two-tailed p-value < 0.05 was regarded as statistically significant. Univariate and multivariate logistic regression analysis were used to test the influence of demographic and clinical parameters such as donor hypertension, acute rejection, and recipient age on the pathogenesis of CAF and mismatches at human leukocyte antigen (HLA) class I (HLA-A or HLA-B) and Class II (HLA-DR or HLA-DQ) loci, between donor and recipient pair with IF/TA sores was calculated using SPSS Statistics software (IBM Corp., Armonk, NY, USA) package.

Results

Demographic profile and clinical characteristics of subjects

All recruited patients in this study, received their kidney transplants between 1997 and 2006. TSB was collected at 224 ± 66 days post-transplantation. The patients′ mean age at the time of the TSB was 47 ± 13.1 years (12–79 years). Of the 218 patients, 57% were men and 43% were women. The majority of the recipients′ ethnicity was Caucasian (71%) followed by Asian (8%) (Table 3). The primary disease in the patients was diabetes or hypertension with a prevalence of 28%. The mean donor age was 36 ± 17 years, and the mean cold ischemia time was 10 ± 7 h. The majority of kidney patients (69%) received grafts from deceased donors. The number of HLA class I (HLA-A, HLA-B) mismatches was 59% and for HLA class II (HLA-DR or HLA-DQ) was 63% (Table 3).

Histologic parameters of TSBs

Eleven histologic parameters were scored in 184 patients who were followed for 6 to 12 months for TSB results (Table 4). The majority of the patients had a histologic grade score ≥ 1. IF/TA, which was calculated based on individual scoring of interstitial fibrosis and tubular atrophy, was observed in 64% of the patients. Other histologic parameters that scored a grade of more than or equal to 1 were 64% tubular atrophy, 58% mesangial matrix, and 51% interstitial fibrosis. The remaining histologic parameters with a score of more than or equal to 1 were observed in 19–43% of patients except for chronic allograft cg and v. The last two parameters had a normal score of 0 in 99% of patients and therefore, were excluded from the analysis (Table 4). Univariate logistic regression analysis of the clinical variables, donor age, donor sex, donor hypertension, and mismatches at HLA class I and class II loci, between patient

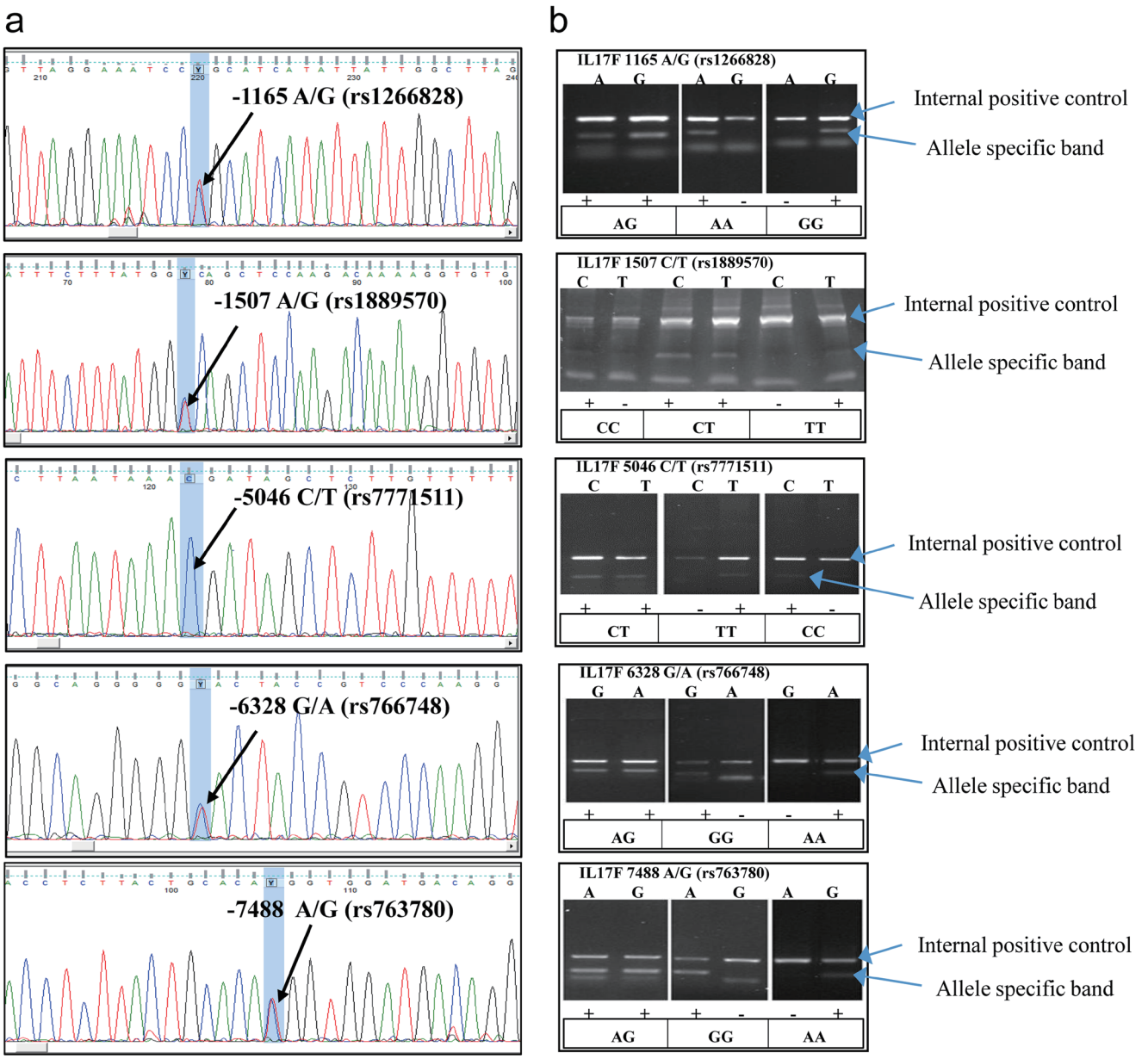

Fig. 1. Representative electropherogram of validation of PCR–SSP-based genotyping through direct sequencing. (a) Representative electropherogram of *IL17F* gene polymorphism using Direct Sequencing. (b) PCR-SSP based genotyping of *IL17F* gene polymorphism.

and donor was significantly associated with IF/TA (Table 5). A significant correlation was observed between an inflammatory grade ≥ 1 and the number of HLA mismatches between the recipient and donor, the episode of acute rejections, and the type of immunosuppressant (Table 5).

Association of IL17F variants with changes in histopathological parameters

A total of five single nucleotide variants of *IL17F* gene were analyzed by SSP–PCR based genotyping in 218 renal transplant patients. To determine the accuracy of the PCR–SSP based typing, DNA from 10 healthy individuals was obtained and genotyped by direct sequencing (Fig. 1). Homozygotes and heterozygotes for each of the *IL17F* variants were then confirmed by PCR–SSP based genotyping.

All the renal transplant patients were scored for 12 histologic parameters for 6–12 months post-transplantation (Table 4). These scores were then tested for their association with the studied *IL17F* variants through univariate logistic regression analysis. Of the studied parameters, the incidence of glomerular sclerosis was found to be associated with *IL17F* 1165 G allele [$p = 0.004$, odds ratio (OR) = 0.39, 95% confidence interval (CI) = 0.21–0.74] and 1165 AA genotype ($p = 0.017$, OR = 2.7, 95% CI = 1.1–6.0). No association was observed between *IL17F 1165* alleles and/or genotypes with any of the histologic parameters. Statistically significant associations of IF/TA, and tubular atrophy were observed with

Table 2. Primers for allele-specific amplification of *IL17* SNPs

Primer name	Primer sequence
1507 Primer forward C	5′-GTAAATCAAAGAATTTCTTTATGGC-3′
1507 Primer forward T	5′-GGTAAATCAAAGAATTTCTTTATGGT-3′
1507 Primer reverse	5′-TCATCTAACATCACCCCCCAC-3′
1165 Primer forward	5′-CCATTGCTATATGCCATGAACCT-3′
1165 Primer reverse T	5′-GAAAACAGGGGTTAGGAAATCCT-3′
1165 Primer reverse C	5′-GAAAACAGGGGTTAGGAAATCCC-3′
5046 Primer forward C	5′-AATCTGTATAAGAAAAATAGAGGCTTAATAAAC-3′
5046 Primer forward T	5′-AATCTGTATAAGAAAAATAGAGGCTTAATAAAT-3′
5046 Primer reverse	5′-GGGTGGCTCCGAAGAAGG-3′
6328 Primer forward	5′-GCCCCATAGTAAGTCTTAATAAACTCATCC-3′
6328 Primer reverse C	5′-ATGAGAAAACCTTGGGACGGTACTG-3′
6328 Primer reverse T	5′-ATGAGAAAACCTTGGGACGGTACTA-3′
7488 Primer forward T	5′-GGATATGCACCTCTTACTGCACTT-3′
7488 Primer forward C	5′-GGATATGCACCTCTTACTGCACTC-3′
7488 Primer reverse	5′-CACCAAGGCTGCTCTGTTTCTT-3′

IL-17, interleukin 17; SNP, single nucleotide polymorphism.

IL17F -1507C allele ($p = 0.029$, OR = 2.2, 95% CI = 1.09–4.47). Moreover, *IL17F -1507T* allele (*rs1889570*) was observed to be associated with better glomerulitis score ($p = 0.041$, OR = 0.3, 95% CI = 0.09–0.93) and allele C of *IL17 -1165C/T* (*rs1266828*) was associated with better glomerular sclerosis ($p = 0.004$, OR = 0.39) score (Table 6).

No other studied variant was found to be associated with any of the histological scores. The associations observed in the present univariate analysis were however, of weak significance and did not yield any significant relationship when corrected for multiple comparisons (data not shown).

Discussion

CAF is a complex inflammatory process that involves HLA, tumor necrosis factor α, transforming growth factor β, and other cytokines. Given that the biologic signature of CAF is an infiltration of inflammatory cells in the renal interstitium, the understanding of the causal agents of CAF is not fully understood. *IL17* cytokine is chiefly produced by Th17 cells and plays an important role in

Table 3. Demographic profile and distribution of clinical characteristics of the studied population

Characteristic	Distribution or range
Number	218
Period of transplantation	1997–2006
Time between transplant and collection of TSB	224 ± 66 days
Recipient	
Age	47 ± 13
Caucasian ethnicity	72%
Male sex	55%
Primary disease of diabetes or hypertension	28%
Donor	
Cadaver	70%
Age	36 ± 17
Cold ischemia time in h	10 ± 7
Number of HLA class I mismatches > 0	59%
Number of HLA class II mismatches > 0	63%

HLA, human leukocyte antigen; TSB, transplant surveillance biopsy.

Table 4. Histological parameters of post-transplant surveillance biopsies obtained at 6–12 months post transplantation

Histological parameter	Patients with grade/ score ≥ 1, % (*n*)	Patients with grades/scores of 0–3, *n*			
		Normal (0)	Mild (1)	Moderate (2)	Severe (3)
Interstitial fibrosis/tubular atrophy (IF/TA)	63.8 (139)	79	122	14	3
Tubular atrophy (ct)	63.8 (139)	79	122	14	3
Mesangial matrix increase (mm)	57.8 (126)	92	77	34	15
Interstitial fibrosis(ci)	50.5 (110)	108	88	18	4
Fibrous intimal thickening (cv)	42.9 (93)	124	74	19	0
Arteriolar hyaline thickening (ah)	41.4 (90)	128	61	23	6
Glomerular sclerosis (gs)	37.6 (82)	136	47	20	15
Interstitial inflammation (i)	30.3 (66)	152	56	7	3
Tubulitis (t)	21.1 (46)	172	21	22	3
Glomerulitis (g)	19.3 (42)	176	30	12	0
Allograft glomerulopathy (cg)	0.9 (2)	216	2	0	0
Intimal arteritis (v)	0.5 (1)	217	1	0	0

the emergence of inflammatory disorders.[19–21] The hypothetical framework of the present study was that, *IL17*, an important pro-inflammatory cytokine, known to have roles in genetic predisposition to a host of inflammatory diseases may be involved in the development of CAF. Five single nucleotide variants of exonic and regulatory regions of *IL17F* genes were investigated for their association with histological parameters related to CAF. The studied gene variants included *IL17F -1507C/T* (*rs1889570*), *-1165A/G* (*rs1266828*), *-5045C/T* (*rs7771511*), *-6328C/T* (*rs766748*) and *-7488G/A* (*rs763780*). As far as is known, this is the first study evaluating the roles of these variants in genetic predisposition to CAF in kidney transplant recipients from America and North America. Our current study did not rely solely on a single pathologist's clinical diagnosis; rather, the association analysis was conducted utilizing specific histological scores that were accurately evaluated by a second pathologist. In the present study, univariate logistic regression analysis showed a weak association of alleles and genotypes of *IL17F* -1165 and alleles of -1507 with glomeru-

Table 5. Significance of the demographical and clinical variables using univariate logistic regression analysis

Clinical variable	Distribution	Code	Dependent variable, *p*-value[a]	
			IF/TA, yes, no	Inflammation, yes, no
Recipient age	≤ 50 year = 133; 50 > year = 87	≥50	0.860	0.941
Donor age	≤ year = 209; > 50 year = 49	≥50	0.001[b]	0.460
Donor type	CAD = 152; living = 66	CAD, living	0.014[b]	0.525
Donor BMI	≤ 30 = 186; > 30 = 25	≥30	0.403	0.465
Donor hypertension	Yes = 23; no = 195	Yes, no	0.011[b]	0.619
Cold ischemia time	≤ 18 h = 177; > 18 h = 34	≥18 h	0.917	0.848
Mismatch number	≤ 2 = 35; > 2 = 183	2 and more	0.092	0.044[b]
Mismatch class 1 (HLA-A or HLA-B)	0 = 10, 1 = 16, 2 = 50, 3 = 60, 4 = 70	0,1,2,3,4	0.047[b]	0.375
Mismatch class 2 (HLA-Dr or HLA-DQ)	0 = 19, 1 = 21, 2 = 72, 3 = 47, 4 = 47	1,2,3,4	0.034[b]	0.242
Acute rejection	Yes = 54; no = 164	Yes, no	0.090	0.000[b]
Delayed graft function	Yes = 13; no = 202	Yes, no	0.673	0.207
CMV infection	Yes = 33; no = 185	Yes, no	0.987	0.684
Immunosuppressant	CsA = 119; FK506 = 99	CsA vs. FK506	0.972	0.000[b]
Induction therapy	Yes = 36; no = 182	Yes, no	0.251	0.253
IL-2 therapy	Yes = 99; no = 119	Yes, no	0.750	0.993

a. Calculated by Fisher's exact test; [b]Values are significant at $p < 0.05$. BMI, body mass index; CAD, Cadaver; CMV, cytomegalovirus; HLA, human leukocyte antigen; IF/TA, interstitial fibrosis/tubular atrophy; *IL*, interleukin.

Table 6. Association between *IL17F* gene polymorphisms and histologic parameters.

Parameters	Values	TT	TC	CC	C	T
IL-17 -1165C/T (rs1266828)						
gs	Frequency (with gs)	33/65 (50.8%)	20/65 (30.8%)	12/65 (18.5%)	32/65 (49.2%)	53/65 (81.5%)
	Frequency (without gs)	34/117 (29.1%)	50/117 (42.7%)	33/117 (28.2%)	83/117 (70.9%)	84/117 (72%)
	p-value		0.017	0.004		NS
	OR (95% CI)		2.7 (1.1–6.0)	0.39 (0.21–0.74)		NS
IL17 -1507C/T (rs1889570)						
IF/TA	Frequency (with IF/TA)	20/119 (16.8%)	90/119 (75.6%)	9/119 (7.6%)	99/119 (83.2%)	110/119 (92.5%)
	Frequency (without IF/TA)	21/68 (30.9%)	42/68 (61.7%)	5/68 (7.3%)	47/68 (69.1 %)	63/68 (92.6%)
	p-value	NS	NS	NS	0.03	NS
	OR (95% CI)	NS	NS	NS	2.2 (1.09–4.47)	
ct	Frequency (with ct)	20/119 (16.8%)	90/119 (75.6%)	9/119 (7.6%)	99/119 (83.2%)	110/119 (92.5%)
	Frequency (without ct)	21/68 (30.9%)	42/68 (61.7%)	5/68 (7.3%)	47/68 (69.1%)	63/68 (92.6%)
	p-value	NS	NS	NS	0.029	NS
	OR (95% CI)	NS	NS	NS	2.2 (1.09–4.47)	NS
g	Frequency (with g)	10/38	22/38	6/38	28/38 (73.7%)	32/38 (84.2%)
	Frequency (without g)	31/149	110/149	8/149	118/149 (83.7%)	141/149 (94.6%)
	p-value	NS	NS	NS	NS	0.041
	OR (95% CI)	NS	NS	NS	NS	0.30 (0.09–0.93)

CI, confidence interval; ct, tubular atrophy; g, glomerulitis; gs, glomerular sclerosis; IF/TA, interstitial fibrosis/tubular atrophy; *IL17*, interleukin-17; NS, not significant; OR, odds ratio.

lar sclerosis as well as interstitial fibrosis/tubular atrophy and glomerulitis respectively. Although a trend was observed with these variants, however, upon correction for multiple comparisons, no association was observed.

IL17 is a proinflammatory cytokine that contributes significantly in tissue inflammation by inducing the release of mobilizing cytokines. Uncontrolled Th17 lineage is known to play a pathogenic role in the chronic inflammation process of autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and psoriasis. It has been demonstrated that ectopic expression of *IL17F* aggravates pulmonary neutrophilia and amplifies inflammatory responses in animal model.[22] Genetic variants such as 6328G/A and 7488A/G, have been found to be associated with chronic inflammatory diseases, particularly atrophic gastritis, rheumatoid arthritis, and myocardial infarction.[15,23,24]

The results of the present study did not support the association of *IL17* polymorphisms with the histopathology associated with CAF, suggesting that the role of these cytokines is more complicated than currently understood in this multifactorial anomaly. In an earlier study from our group,[7] we reported that high IF/TA grade and interstitial inflammation scores, as well as a significant influx of inflammatory cells in the renal allograft interstitium, were all highly related to kidney transplant patients who were genetically predisposed to produce low levels of *IL10* and no association of low production of *IL10* was observed with other tested histologic parameters.

Recently, gene polymorphisms in *IL17A* and *IL17F* were associated with histopathological changes in kidney transplants.[25] Authors reported a possible association between the *IL17A* gene polymorphisms and histopathological changes in biopsies. A weak association was reported with the C allele at the -7488 A/G *IL17F* gene polymorphism with IF/TA score, however, no significant association remained significant after multiple-level correction. Similar to the finding we also observed a weak association of C allele of *IL17 -1507C/T* (*rs1889570*) with IF/TA score ($p = 0.03$) (Table 6). Another paper from the group of Tunisia examines the effects of *IL17F* gene polymorphisms on kidney transplantation outcomes utilizing direct sequencing in 93 recipients of kidney transplants.[26] The *IL17F* SNPs under study did not show any statistically significant correlation with the onset of acute rejection. They concluded that the *IL17F* 7489A/G A allele may be associated with a decreased risk of acute rejection and improved graft survival, as in the present study allele T of *IL17 -1507C/T* (*rs1889570*) was associated with the better glomerulitis score ($p = 0.041$, OR = 0.30) and allele C of *IL17-1165C/T* (*rs1266828*) was associated with better glomerular sclerosis ($p = 0.004$, OR = 0.39) (Table 6). Further, another study, published by Hejr *et al.*,[27] on the association of *IL17* gene polymorphisms with HBV-induced rejection in kidney transplants, is also in favor of our study. The studied gene polymorphism of *IL17* (*-197 A/G*, *rs2275913*) did not associate with acute rejection. On the other hand, Romanowski *et al.* studied the impact of *IL17A* and *IL17F* gene polymorphisms on post-kidney transplant return to dialysis and long-term kidney allograft function in 269 Caucasian deceased donor renal transplant recipients.[17] Genotyping was done on the *IL17A* and *IL17F* gene polymorphism and creatinine concentrations as a cutoff criterion for transplant rejection detection. None of the genotypes of *IL17F* was directly associated with the delayed graft function. In multivariate logistic regression analysis adjusted for recipients′ age and sex, they observed an association between the C allele and decreased probability of delayed graft function (OR = 0.44, $p = 0.046$). They further concluded that the GA genotype of the *IL17F* gene polymorphism

(*rs11465553*) may be linked to a risk of graft function loss and a need for dialysis again following kidney transplantation.

Even though there is some experimental evidence directly associating Th17 cells with allograft rejection, the majority of clinical transplantation studies that have been published so far are concerned with *IL17* expression and *IL17* serum level.[28,29] A study by Crispim *et al.* on kidney allograft outcome reported significantly increased levels of *IL17* among samples derived from patients with rejection in contrast,[30] to the nonrejection group. Studies have shown that *IL17* is involved in experimental and human renal allograft rejection at an early stage, and human subclinical rejection allograft biopsy tissue has been reported to exhibit *IL17* protein.[11] Yuan *et al.* have demonstrated the importance of CD4+ Th17 cells for allograft rejection where an aggressive proinflammatory response leading to severe accelerated allograft rejection and vasculopathy was detected in CD4+ Th17 mediated cells.[31] There is very little data or no study available on the expression profile of *IL17* and delayed graft function compared to allograft rejection.

The absence of significant associations with chronic kidney allograft failure in this study could be the smaller sample size in genotype distribution and correlation with CAF which is one of the limitations of the study. Although more than 70% of the renal transplant recipients included in the study were Caucasians, a potential bias of demographic stratification may also be seen as a limiting factor. This study did not examine the role of *IL17* polymorphism in donors and because of the lack of availability of serum at different time points, we were unable to detect the serological levels of *IL17* in CAF which is another limiting factor. Because many patients were lost during follow-up, we were unable to do the survival analysis. Studies on hematopoietic stem transplant setting have shown that high-producing *IL17* polymorphism in donors showed a trend of association with acute graft versus host disease.[32]

Conclusions

Our results do not support a major association of *IL17F* SNPs with predisposition to CAF. A lack of association of an important proinflammatory cytokine, *IL17*, in the present study, is consistent with our earlier observation and other studies published online. This emphasizes the nuanced nature of genetic associations and underscores the importance of carefully analyzing and interpreting genetic data while accounting for statistical considerations. Unfortunately, there are few studies on the effect of *IL17* gene polymorphisms in chronic kidney allograft failure, which makes it challenging to compare the data. Therefore, more studies from different parts of the world and North America are needed to confirm these findings.

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Conflict of interest

The authors declare that they have no conflict of interests related to this study.

Author contributions

Conception and study design (NB, FK and AL); data generation and analysis (GT, AL, FK and DIO); original manuscript draft and revision (GT and AL); scientific input (NB, AL, GT and FK); performed the study (DIO and AL); provided materials and reagents, and managed the study (AS, SY, NB, FK); revision of manuscript (GT, AL, DIO, RF, FK, AS, SY and NB). All authors have read and approved the manuscript.

Ethical statement

All procedures performed in studies involving human participants were in accordance with the Helsinki declaration or comparable ethical standards. Ethical clearance (ethics ID 24259) was obtained from University of Calgary, Calgary, Canada, and written informed consent was obtained from all individual participants included in the study.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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