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STAT4 gene polymorphism in patients after renal allograft transplantation

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Abstract

Introduction: *STAT4 (signal transducer and activator of transcription 4) is involved in the regulation of innate and adaptive immune responses. Some studies have suggested that STAT4 may be involved in the immune response after graft transplantation. Several polymorphisms in the STAT4 gene have been identified. The most commonly studied polymorphism in the STAT4 gene is rs7574865. In our study, we examined whether this polymorphism is associated with the early and late functions of renal allografts.*

Material and methods: *A total of 270 recipients of first renal transplants were included in the study. Single nucleotide polymorphisms (SNPs) within the STAT4 gene were genotyped using TaqMan genotyping assays.*

Results: *There were no statistically significant associations between the STAT4 gene rs7574865 polymorphism and delayed graft function, acute rejection, chronic allograft dysfunction, post-transplant diabetes mellitus, or creatinine serum concentrations after transplantation.*

Conclusions: *Our results suggest a lack of association between the STAT4 rs7574865 SNP and kidney allograft function in the Polish population.*

Key words: *STAT4, polymorphism, kidney, graft.*

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Introduction

STATs (signal transducers and activators of transcription) are DNA-binding transcription factors that induce the transcription of genes by recognising specific DNA sequences. There are seven different members of the STAT family (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6), which are involved in the regulation of the innate and adaptive immune responses [1]. STAT4 protein comprises an N-terminal domain that plays an important role in phosphorylation and nuclear translocation, as well as a four-stranded helical coiled coil that is implicated in protein–protein interaction and nuclear import and export [2]. STAT4 plays a significant role in the regulation of natural killer (NK) cells, CD8+ T cells, and Th1 function, as well as in the differentiation of B cells and regulatory T (Treg) cells [3, 4]. STAT4 is expressed in activated peripheral blood monocytes, dendritic cells, and macrophages, and plays a significant role in the signalling pathway of several important cytokines [5, 6]. Furthermore, dysregulation of STAT4 has been found in some autoimmune diseases [7-9].

STAT4 was mapped to chromosome 2q32.2–q32.3 [10]. The most commonly studied polymorphism in the *STAT4* gene is rs7574865 [8, 9, 11]. This polymorphism is located in the third intron of the *STAT4* gene, but its functional consequences are not known. It is possible that the rs7574865 polymorphism influences STAT4 expression or phosphorylation. The *STAT4* gene polymorphism is emerging as a novel risk factor for various diseases, in particular, autoimmune diseases, such as ulcerative colitis, rheumatoid arthritis, diabetes, systemic, lupus erythematosus, and Sjögren's syndrome [7-9, 11]. Some studies have suggested that STAT4 may be involved in the immune response after graft transplantation [12]. In our study, we examined whether *STAT4* rs7574865 polymorphism is associated with the early and late functions of renal allografts.

Material and methods

A total of 270 recipients of first renal transplants were included in the study (166 males, 104 females, mean age 47.63 ±12.96 years; transplantation performed between 2001 and 2009 in the Clinical Department of Nephrology

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and Transplantology of the Pomeranian Medical University in Szczecin, Poland). The main causes of chronic kidney disease and renal transplantation were: glomerulonephritis (58%), hypertension (9%), and diabetes (9%). The histories of the patients were analysed, taking into account delayed graft function (DGF), acute rejection, and chronic allograft dysfunction. Delayed graft function was defined as the need for haemodialysis within the first seven days after transplantation. Acute rejection episodes were defined by clinical diagnosis (elevated serum creatinine in the absence of other pathology, including infection, urinary tract obstruction, allograft artery stenosis, or cyclosporine toxicity) and were confirmed by positive biopsy. Chronic allograft dysfunction was diagnosed by eliminating other causes, including infections, urinary obstruction, allograft artery stenosis, or cyclosporine toxicity, and by changes in biopsy samples. This process was diagnosed clinically in patients that had a slow persistent rise in serum creatinine of at least 30% above baseline, usually accompanied by new or worsening hypertension and proteinuria (above 500 mg/24 h). Anatomical problems were excluded by ultrasound and nuclear scans. Biopsy criteria included the presence of interstitial fibrosis, tubular atrophy, and, particularly, characteristic vascular changes, such as hypertrophy of the arterial intima and smooth muscle (intimal thickening) and glomerular sclerosis. All biopsies were reviewed by a renal pathologist, and the Banff working classification criteria were used in the histological classification of the biopsies [13]. Blood samples were collected from all patients for genetic analysis at the start of the study and for the evaluation of creatinine concentration at 1, 3, 6, 12, 24, and 36 months after kidney transplantation. Creatinine concentration was measured using a colorimetric method. Patients with haemoglobin A1c continuously over 6.5%, fasting blood glucose ≥ 7.0 mmol/l, or requiring treatment with oral hypoglycaemic agents or insulin for more than three months after transplantation were diagnosed as having post-transplant diabetes mellitus (PTDM) [14].

All patients received a standard immunosuppressive protocol with triple-drug therapy, including calcineurin inhibitors (cyclosporine A in 75% and tacrolimus in 24% of recipients), azathioprine (55%) or mycophenolate mofetil (37%), and steroids (91%). The study was approved by the Ethics Committee in Pomeranian Medical University, Szczecin, Poland, and written informed consent was obtained from all subjects.

Genotyping

SNPs within the *STAT4* gene were genotyped using TaqMan genotyping assays. Genomic DNA was extracted from 200 µl of whole blood samples using a GeneMATRIX Quick Blood DNA Purification Kit (EURx, Poland). Fluorescence data was captured using a 7500 FAST Real-Time PCR System (Applied Biosystems, USA).

Statistical analysis

Chi-square test and Fisher's exact test were used to compare genotype and allele frequencies between groups. The serum concentrations of creatinine were compared between genotype groups using the non-parametric Kruskal-Wallis test because their distributions were significantly different from normal. A p-value < 0.05 was considered statistically significant.

Results

The GG genotype was diagnosed in 58.15% of transplant recipients, GT in 34.44%, and TT in 7.41%. The distribution of the genotypes was in Hardy-Weinberg equilibrium ($p = 0.25$).

Delayed graft function was diagnosed in 34.39% of the patients with the GG genotype, 24.73% with GT, and

Table 1. The association between *STAT4* genotypes and delayed graft function (DGF)

	Without DGF		DGF		*p*-value^		*p*-value*	OR (95% CI)
	n	%	*n*	%				
***STAT4* rs7574865 genotype**								
GG	103	65.61	54	34.39	0.20	TT + GT vs. GG	0.24	0.72 (0.43-1.22)
GT	70	75.27	23	24.73		TT vs. GT + GG	0.45	1.50 (0.59-3.81)
TT	12	60.00	8	40.00		TT vs. GG	0.63	1.27 (0.49-3.30)
						GT vs. GG	0.12	0.63 (0.35-1.11)
						TT vs. GT	0.18	2.03 (0.74-5.58)
***STAT4* rs7574865 allele**								
G	276	74.59	131	77.06		T vs. G	0.59	0.87 (0.57-1.34)
T	94	25.41	39	22.94				

^χ^2 test; *Fisher's exact test

Table 2. The association between *STAT4* genotypes and acute rejection

	Without acute rejection		Acute rejection		*p*-value^		*p*-value*	OR (95% CI)
	n	%	*n*	%				
***STAT4* rs7574865 genotype**								
GG	115	73.25	42	26.75	0.51	TT + GT vs. GG	0.78	0.90 (0.52-1.57)
GT	68	73.12	25	26.88		TT vs. GT + GG	0.30	0.48 (0.14-1.70)
TT	17	85.00	3	15.00		TT vs. GG	0.41	0.48 (0.13-1.73)
						GT vs. GG	1.00	1.01 (0.56-1.80)
						TT vs. GT	0.39	0.48 (0.13-1.78)
***STAT4* rs7574865 allele**								
G	298	74.50	109	77.86		T vs. G	0.49	0.83 (0.53-1.31)
T	102	25.50	31	22.14				

^ χ^2 *test;* * *Fisher's exact test*

Table 3. The association between *STAT4* genotypes and chronic allograft dysfunction

	Without chronic allograft dysfunction		Chronic allograft dysfunction		*p*-value^		*p*-value*	OR (95% CI)
	n	%	*n*	%				
***STAT4* rs7574865 genotype**								
GG	123	78.34	34	21.66	0.43	TT + GT vs. GG	0.56	1.19 (0.67-2.11)
GT	68	73.12	25	26.88		TT vs. GT + GG	0.58	0.57 (0.16-2.02)
TT	17	85.00	3	15.00		TT vs. GG	0.77	0.64 (0.18-2.31)
						GT vs. GG	0.36	1.33 (0.73-2.41)
						TT vs. GT	0.39	0.48 (0.13-1.78)
***STAT4* rs7574865 allele**								
G	314	75.48	93	75.00		T vs. G	0.91	1.03 (0.65-1.63)
T	102	24.52	31	25.00				

^ χ^2 *test;* * *Fisher's exact test*

Table 4. The association between *STAT4* genotypes and post-transplant diabetes mellitus (PTDM)

	PTDM absent		PTDM present		*p*-value^		*p*-value*	OR (95% CI)
	n	%	*n*	%				
***STAT4* rs7574865 genotype**								
GG	81	84.38	15	15.63	0.67	TT + GT vs. GG	0.50	0.66 (0.27-1.66)
GT	55	88.71	7	11.29		TT vs. GT + GG	1.00	0.62 (0.08-5.07)
TT	10	90.91	1	9.09		TT vs. GG	1.00	0.54 (0.06-4.54)
						GT vs. GG	0.49	0.69 (0.26-1.80)
						TT vs. GT	1.00	0.79 (0.09-7.10)
***STAT4* rs7574865 allele**								
G	217	74.32	37	80.43		T vs. G	0.46	0.70 (0.32-1.53)
T	75	25.68	9	19.57				

^ χ^2 *test;* * *Fisher's exact test*

Table 5. The association between *STAT4* genotypes and serum creatinine after transplantation

Creatinine (mg/dl)	*STAT4* rs7574865 genotype						*p*-value[#]
	GG		GT		TT		
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	
1 month	157	1.89 ±0.99	93	1.84 ±0.72	20	1.67 ±0.51	0.81
3 months	157	1.77 ±0.61	93	1.75 ±0.62	20	1.76 ±0.52	0.83
6 months	157	1.77 ±0.65	92	1.81 ±0.67	20	1.72 ±0.39	0.93
12 months	153	1.76 ±0.59	91	1.79 ±0.68	20	1.67 ±0.45	0.93
24 months	148	1.72 ±0.55	90	1.79 ±0.69	18	1.69 ±0.57	0.85
36 months	139	1.73 ±0.55	83	1.71 ±0.65	18	1.75 ±0.79	0.72

[#]*Kruskal-Wallis test*

40.00% with TT. The differences were not statistically significant (Table 1).

Acute rejection was diagnosed in 26.75% of the carriers of the GG genotype, 26.88% of GT carriers, and 15.00% of those with TT. The differences were not statistically significant (Table 2).

Chronic allograft dysfunction was diagnosed in 21.66% of the patients with the GG genotype, 26.88% with GT, and 15.00% with TT. The differences were not statistically significant (Table 3).

Post-transplant diabetes mellitus was diagnosed in 15.63% of the patients with the GG genotype, 11.29% with GT, and 9.09% with TT. The differences were not statistically significant (Table 4).

Creatinine concentrations at 1, 3, 6, 12, 24, and 36 months after transplantation did not differ significantly between the different genotypes of the *STAT4* gene rs7574865 polymorphism (Table 5).

Discussion

In this study we analysed the association between the *STAT4* gene rs7574865 polymorphism and kidney allograft function. We examined the association between the *STAT4* gene rs7574865 polymorphism and DGF, acute rejection, chronic allograft nephropathy, post-transplant diabetes, and creatinine concentrations up to 36 months after transplantation. There were no statistically significant associations between this polymorphism and early and late graft function.

STAT4 plays a crucial role in the function of innate and adaptive immune cells [15]. Moreover, dysregulated expression and aberrant activation of STAT4 is observed in many human autoimmune diseases. STAT4 also plays a significant role in the immune response after transplantation. STAT4 is critical in Th-cell activation. Chang *et al*. [16, 17] revealed that STAT4 deficiency prevented development of Th1 cells from post-transplant patients, and this correlated with reduced interferon γ (IFN-γ) in Th1 cells.

Only a few studies have examined the role of STAT4 in the immune response after allograft transplantation. Liang *et al*. analysed the various IFN-γ-dependent functions in terms of Th1 and Th2 responses during rejection. These authors investigated mice deficient in the transcription factors STAT4 and IFN-γ. The data showed that the STAT4-deficient groups have significantly prolonged graft survival [18].

Zhou *et al*. investigated the role of STAT4 signalling in allograft rejection and CTLA4-Ig-mediated tolerance [19]. *STAT4*(–/–) mice have impaired type 1 T-cell differentiation. The role of Th1 and Th2 cell differentiation in acute cardiac allograft rejection and in the induction of tolerance was examined in wild-type and *STAT4*(–/–) recipients. Analysis of in situ cytokine gene expression in the allografts confirmed decreased levels of IFN-γ in *STAT4*(–/–) recipients. Blockade of the CD28/B7 co-stimulatory pathway prolonged cardiac graft survival in 100% of wild-type and *STAT4*(–/–) mice. These data suggest that the balance of Th1 and Th2 differentiation is not critical for acute rejection but does influence the tolerance induced by CD28/B7 blockade.

Eurich *et al*. evaluated the role of the *STAT4* rs7574865 polymorphism in the development of HCV-related graft disease based on protocol biopsies [20]. During an observation period, 46.5% of patients developed advanced fibrosis. Advanced fibrosis was observed significantly more frequently in patients with at least one T allele compared with homozygotes for the G allele. Significant differences in the duration of advanced fibrosis development were detected between patients with at least one T allele compared with the G allele. No impact was observed regarding the outcome of interferon-based antiviral treatment and the occurrence of acute cellular rejection. These results indicate a possible impact of the *STAT4* T allele on graft fibrogenesis, thus explaining the significantly different graft behaviour observed after transplantation for HCV-associated liver disease.

The *STAT4* rs7574865 polymorphism has been correlated with diabetes susceptibility. Members of the STAT family are transcription factors that mediate the signalling events of many cytokines in immune and non-immune cells [3, 21]. STAT4 is also involved in the development

of a newly discovered subset of Th17 cells, which display a dominant role in autoimmunity-associated inflammation, including diabetes [22]. Results from recent studies have indicated an association between the rs7574865 single-nucleotide polymorphism in the *STAT4* gene and insulin resistance and diabetes risk, especially diabetes type I [23-25]. In our study we analysed the association between the *STAT4* rs7574865 polymorphism and PTDM. Our results did not reveal any significant association between this polymorphism and PTDM.

Bolin *et al*. examined *STAT4* polymorphisms in patients with lupus nephritis in two cohorts of Swedish patients. These authors have indicated that the *STAT4* rs7582694 polymorphism may predispose to lupus nephritis and a worse outcome of this disease with severe renal insufficiency [26]. Only one study has examined the role of the *STAT4* rs7574865 polymorphism in kidney allograft function. Yang *et al*. evaluated the association between the *STAT4* rs7574865 polymorphism and acute allograft rejection in the Chinese population [27]. No evidence of an association was found between healthy controls and renal transplant recipients for the GT or TT genotype and the wild-type GG. However, among the transplant recipients, the GT or TT genotype was more common in patients with acute allograft rejection than in those without rejection who mostly had the wild-type GG genotype. Thus, the authors suggested that the *STAT4* rs7574865 SNP is a genetic susceptibility variant for acute renal allograft rejection in the Chinese population.

Among our transplant recipients, there was no statistically significant association between the *STAT4* rs7574865 SNP and acute allograft rejection. This lack of association may be caused by ethnic differences. Our patients were from a Caucasian population with different genetic backgrounds than the Chinese population. Our results suggest a lack of association between the *STAT4* rs7574865 SNP and kidney allograft function in the Polish population. Nevertheless, the role of STAT4 in immunity and allograft function requires further investigation.

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