Interleukin-4 (*IL4*) -590C/T (rs2243250) gene polymorphism is not associated with diabetic nephropathy (DN) in Caucasians with type 2 diabetes mellitus (T2DM)

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ABSTRACT

Diabetic nephropathy (DN) is a microvascular complication that affects up to 40% of diabetic patients and can lead to end-stage kidney disease. Inflammatory cytokines such as interleukin 1 (IL-1), IL-6, IL-18 and tumor necrosis factor-α (TNFα) have been linked to the development and progression of DN. The aim of our study was to examine the relationship between interleukin-4 (*IL4*) -590C/T (rs2243250) gene polymorphism and DN in patients with type 2 diabetes mellitus (T2DM). This study is a continuation of our previous research on the association between angiotensinogen (*AGT*) gene polymorphisms and DN in patients with T2DM. We included 651 unrelated Slovenian (Caucasian) patients who had had T2DM for at least 10 years. The participants were classified into a group of T2DM patients with DN (276 cases) and a group without DN (375 controls). *IL4* rs2243250 polymorphism was analyzed using a TaqMan SNP genotyping assay and StepOne Real-Time PCR System. The frequencies of rs2243250 TT, CT and CC (wild type) genotypes were 3.2%, 29.4% and 67.4%, respectively in patients with DN, and 2.7%, 34.4% and 62.9%, respectively in controls. Our logistic regression analysis adjusted for gender, age, diabetes duration, and glycated hemoglobin showed no association between rs2243250 and the risk for DN (OR 1.06; CI 0.37-3.05; $p = 0.9$). *IL4* rs2243250 is not associated with DN in our subset of Slovenian patients with T2DM.

KEY WORDS: Interleukin 4; rs2243250; diabetic nephropathy; association study
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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia which develops as the result of reduced insulin secretion and/or ineffectiveness of produced insulin. Global prevalence of diabetes mellitus (DM) in adults aged 20 to 79 years is estimated to rise from 8.8% in 2015 to 10.4% by the year 2040 [1]. Moreover, in the United States alone, a 165% increase in the prevalence of DM is expected between 2000 and 2050 [2].

Complications of diabetes can be classified as macrovascular and microvascular. A common microvascular complication is diabetic nephropathy (DN), a kidney disease defined by the presence of abnormal levels of urinary albumin and/or decreased glomerular filtration rate (GFR) that affects up to 40% of all patients with diabetes [3]. Patients with DN have a higher mortality rate and are more prone to cardiovascular diseases (CVDs) compared to diabetic patients without DN [4].

Familial aggregation of albuminuria, end-stage kidney disease and chronic kidney disease, as well as differences in the prevalence of DN between populations, suggest a strong genetic component of DN [5]. Thus, identification of genes that might be involved in the onset and progression of DN is important for the development of new preventive and therapeutic strategies.

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Immune-mediated inflammatory processes have been implicated in the pathophysiology of diabetes and its complications, and a number of studies investigated the specific roles of inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-18, and tumor necrosis factor-α (TNFα) in the development of DN [6].

IL-4 is an anti-inflammatory cytokine involved in the regulation of the immune system at different levels. For example, IL-4 stimulates the proliferation of activated T and B cells, regulates the differentiation of B cells, promotes type 2 T helper (Th2) and inhibits type 1 T helper (Th1) cell differentiation [7-10]. In addition to lymphoid cells, IL-4 is able to modulate the differentiation, proliferation and apoptosis of other hematopoietic as well as non-hematopoietic cell populations [11].

IL-4 and the related signaling pathways have been linked to the development of autoimmune [12] and allergic diseases [13]. For instance, an association of allergic asthma with IL-4 was clinically demonstrated by a significant increase in airway hyperresponsiveness in patients with mild asthma after administration of IL-4 with a nebulizer [13]. Generally, it is suggested that allergic diseases are dependent on Th2 cells and related cytokines, whereas autoimmune diseases depend on Th1 cells and cytokines produced by monocytes [12]. The anti-inflammatory role of IL-4 in autoimmune diseases has been indicated based on its protective effects in murine models of diabetes and rheumatoid arthritis [11,14].

The *IL4* gene, located at the long arm of chromosome 5 (5q31), is 0.9 kb long and contains 4 exons [15]. Several studies have shown that a polymorphism in the promoter region of *IL4* (*IL4* -590C/T, rs2243250) might be associated with genetic susceptibility to atopic dermatitis [16], multiple sclerosis, rheumatoid arthritis [17], and atopic asthma [18].

In this study, we investigated the relationship between *IL4* -590C/T (rs2243250) polymorphism and DN in patients with type 2 diabetes mellitus (T2DM).

MATERIALS AND METHODS

Patients

This study is a continuation of our previous research on the association between angiotensinogen (*AGT*) gene polymorphisms and DN in patients with T2DM [19]. We included 651 unrelated Slovenian (Caucasian) patients who had had T2DM for at least 10 years. The participants were classified into a group of T2DM patients with DN (276 cases) and a group without DN (375 controls) [19]. The diagnosis of T2DM and DN was made according to the World Health Organization diagnostic criteria. To avoid the confounding effect of impaired kidney function, patients with overt nephropathy were not enrolled in the study. Additional exclusion criteria were poor glycemic control, significant heart failure [New York Heart Association (NYHA) Classification II-IV], alcoholism, infection, and the presence of other causes of renal disease.

The study was approved by the national medical ethics committee and performed in compliance with the Helsinki declaration. All participants provided informed consent. Information on age, sex, blood pressure, duration of T2DM and hypertension, body mass index (BMI), smoking status, incidence of microvascular complications of T2DM (diabetic retinopathy [DR], DN, diabetic foot [DF]), duration of DR, estimated GFR (eGFR), hemoglobin (Hb), total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels was obtained by questionnaire [19].

Biochemical analyses

Plasma glucose, Hb, glycated hemoglobin (HbA1c), urea, creatinine, cystatin C, total cholesterol, LDLs, HDLs, and triglycerides (TGs) were determined by standard biochemical methods. Albumin/creatinine ratio (ACR) was determined in three urine samples for each patient [19].

Genotyping

Genomic DNA was extracted from 100 µl of peripheral blood using a DNeasy Blood and Tissue Kit (Qiagen, Germany). *IL4* rs2243250 polymorphism was analyzed using a TaqMan single nucleotide polymorphism (SNP) genotyping assay and 48-well StepOne Real-Time PCR System, according to the manufacturer's instructions (Applied Biosystems, USA). The volume of PCR reaction (5 µl) was calculated with StepOne real-time PCR Systems software, and the following components were included in the reaction mix: TaqMan Universal Master Mix, mix of fluorescent dye (VIC/FAM)-labeled oligonucleotide primers, RNase-free H_2O, and 0.5 µl of DNA. The amplification was carried out under the following conditions: step 1, pre-PCR plate read for 30 seconds at 60°C; step 2, pre-denaturation for 10 minutes at 95°C, repeating 35 cycles with denaturation for 15 seconds at 95°C and annealing/extension for 60 seconds at 60°C; step 3, post-PCR plate read for 30 seconds at 60°C. Three control samples with known genotypes and negative control were applied in each series.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp., Armonk, NY). Continuous variables were compared by either unpaired Student's *t*-test or Mann–Whitney *U* test. Chi-square test was used to compare discrete variables. The relationship between *IL4* rs2243250 polymorphism and DN was assessed by logistic regression analysis, adjusted for gender, age, diabetes duration, and HbA1c concentration. The value of $p < 0.05$ was

TABLE 1. Clinical characteristics of T2DM patients with and without DN

Characteristic	Cases (DN+)	Controls (DN-)	*p*
Number	276	375	
Sex, M (%)	59.1	52.4	0.1
Age (years)	64.75±9.15	63.75±8.0	0.13
Duration of T2DM (years)	14.0 (10.0–19.0)	13.5 (11.0–18.3)	0.84
Duration of hypertension (years)	10 (5–17)	10 (4–15)	0.06
SBP (mmHg)	155.27±18.92	149.84±19.63	<0.001
DBP (mmHg)	84.87±11.63	84.06±11.42	0.36
BMI	31.3±4.68	30.77±5.0	0.23
Active smokers (%)	6.6	8.9	0.31
CVD (%)	20.0	12.2	0.007
Family history of CVD (%)	41.3	58.7	0.91
DR (%)	37.8	24.6	<0.001
Duration of DR (years)	3.94±3.11	6.54±7.03	0.23
DNeur (%)	9.1	6.0	0.38
DF (%)	15.5	8.1	0.03

Data are presented as mean±SD, proportions/percentages, or median (IQR) values. T2DM: Type 2 diabetes mellitus; DN: Diabetic nephropathy; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; CVD: Cardiovascular disease; DR: Diabetic retinopathy; DNeur: Diabetic neuropathy; DF: Diabetic foot; IQR: Interquartile range.

TABLE 2. Laboratory characteristics of T2DM patients with and without DN

Characteristic	Cases (DN+)	Controls (DN-)	*p*
Number	276	375	
S-HbA1c (%)	7.98±1.38	7.65±1.14	0.001
S-fasting glucose (mmol/l)	9.03±2.76	8.51±2.53	0.01
S-Hb (g/l)	139.39±14.91	139.40±12.96	0.99
S-urea (mmol/l)	7.35±3.73	6.25±1.91	<0.001
S-creatinine (μmol/l)	81.0 (66.0–103.0)	76.0 (64.0–89.8)	0.002
eGFR (MDRD equation, ml/minute)	72.6±19.74	75.22±15.16	0.22
Male sex	71.97±19.45*	77.66±14.33*	0.002*
Female sex	74.31±20.72**	72.45±15.69**	0.13**
S-cystatin C (mg/l)	0.8 (0.7–1.1)	0.7 (0.6–0.9)	<0.001
S-Total cholesterol (mmol/l)	4.62±1.17	4.55±0.99	0.42
S-HDL (mmol/l)	1.23±0.35	1.26±0.36	0.29
S-LDL (mmol/l)	2.59±0.95	2.57±0.80	0.73
S-TGs (mmol/l)	1.6 (1.1–2.5)	1.5 (1.0–2.3)	0.04
U-albumin/creatinine ratio (g/mol) - sample number 1	9.4 (4.5–33.6)	1.0 (0.6–1.6)	<0.001
U-albumin/creatinine ratio (g/mol) - sample number 2	10.6 (4.5–33.9)	1.0 (0.7–1.7)	<0.001
U-albumin/creatinine ratio (g/mol) - sample number 3	9.5 (4.3–33.9)	1.1 (0.7–1.8)	<0.001

Data are presented as mean±SD, proportions/percentages, or median (IQR) values. T2DM: Type 2 diabetes mellitus; DN: Diabetic nephropathy; HbA1c: Glycated hemoglobin A1c; Hb: Hemoglobin; eGFR: Estimated glomerular filtration rate; MDRD: Modification of Diet in Renal Disease Study; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TGs: Triglycerides; S: Serum; U: Urine; IQR: Interquartile range. *Comparison of men with DN versus men without DN; **Comparison of women with DN versus women without DN.

TABLE 3. Distribution of *IL4*-590C/T (rs2243250) genotypes and alleles in T2DM patients with DN (cases) and without DN (controls)

rs2243250	Cases (276)	Controls (375)	*p*
TT	9 (3.2)	10 (2.7)	
CT	81 (29.4)	129 (34.4)	0.4
CC	186 (67.4)	236 (62.9)	
T allele (%)	99 (17.9)	149 (19.9)	0.4
C allele (%)	453 (82.1)	601 (80.1)	
PHWE†	0.9	0.1	

†P_{HWE} values were computed using Pearson's goodness-of-fit Chi-square (1 degrees of freedom [df]). HWE: Hardy-Weinberg equilibrium; IL-4: Interleukin-4; T2DM: Type 2 diabetes mellitus; DN: Diabetic nephropathy.

considered statistically significant. The deviation from Hardy-Weinberg equilibrium (HWE) was assessed by the Fisher's exact test (http://ihg.gsf.de/).

RESULTS

Demographic and clinical characteristics of T2DM patients with and without DN are summarized in Table 1 and 2. Higher systolic blood pressure, higher ACR, presence of CVD, and increased levels of serum fasting glucose, HbA1c, urea, creatinine, and triglycerides were significantly more

TABLE 4. Association of *IL4*-590C/T (rs2243250) polymorphism with DN in T2DM patients

Inheritance model	rs2243250 genotype	Cases (276)	Controls (375)	Adjusted OR (95% CI)	†*p*
	TT	9 (3.2)	10 (2.7)	1.06 (0.37–3.05)	0.9
Codominant	CT	81 (29.4)	129 (34.4)	0.79 (0.54–1.17)	0.2
	CC	186 (67.4)	236 (62.9)	Reference	

†*p* values were adjusted for gender, age, diabetes duration, and glycated hemoglobin concentration. IL-4: Interleukin-4; T2DM: Type 2 diabetes mellitus; DN: Diabetic nephropathy; OR: Odds ratio; CI: Confidence interval.

frequent in patients with DN. In addition, the prevalence of DR and DF was significantly higher in patients with DN compared to those without DN (Table 1). Similarly, serum cystatin C and creatinine levels were significantly higher in patients with DN (Table 2).

The distribution of *IL4* rs2243250 genotypes and alleles is shown in Table 3. rs2243250 was in HWE in both groups and there was no statistically significant difference in the genotype distribution between T2DM patients with and without DN (Table 3).

A logistic regression analysis was used to evaluate whether rs2243250 polymorphism was independently associated with DN after adjusting for gender, age, diabetes duration, and HbA1c concentration. The results indicated no relationship between rs2243250 polymorphism and DN in our cohort of T2DM patients (Table 4).

DISCUSSION

Chronic inflammation is considered to be the underlying cause of DN. In tissue and organs, different types of immune cells function as internal sensors of damage and thus have a primary role in the process of inflammation [20]. IL-4 is involved in the differentiation of T cells and a change in the gene sequence might affect the anti-inflammatory function of protein, and consequently influence the pathogenesis of DN [9]. This assumption was the basis of our study, however, in our group of patients with T2DM we did not observe an association between *IL4* -590C/T (rs2243250) polymorphism and DN.

Up until now, only two other studies have investigated the association between *IL4* -590C/T polymorphism and DN. Arababadi [21] showed a significant difference in the genotype and allele frequencies of -590C/T SNP between T2DM patients with DN and healthy controls in a Rafsanjan population from the southeast of Iran [21]. Similarly, Neelofar et al. [22] found an association between *IL4* -590C/T and *IL6* -174G/C polymorphisms and chronic kidney disease in North Indian patients with T2DM [22]. In addition, Alsaid et al. [23] indicated that, in their group of Egyptian patients with diabetes, the heterozygous genotypes of *IL4* -590 (CT) and *IL13* -1112 (CT) could be considered as risk factors while the homozygous wild-type genotypes of both genes (CC) might be considered protective for T2DM [23].

Comparable to the results of the current study, Cilenšek et al. showed no association between *IL4* -590C/T SNP and another microvascular complication of T2DM, proliferative DR, in Caucasians with T2DM [24].

We assume that the differences in the frequency of -590C/T alleles between diverse and geographically distant populations (in this case Iranian and Indian versus Slovenian population) might, at least partially, explain the discrepant results and the lack of association between *IL4* rs2243250 and DN in our study.

Overall, we did not find an association between *IL4* -590C/T gene polymorphism and DN in Slovenian (Caucasian) patients with T2DM. This implies that *IL4* rs2243250 may not be a marker for DN in Caucasians with T2DM.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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