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ORIGINAL ARTICLE

Monocyte chemoattractant protein-1 (MCP-1) 2518G/A gene polymorphism in Turkish type 2 diabetes patients with nephropathy

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Abstract Tissue macrophage accumulation is thought to induce insulin resistance during obesity and stimulate the progression of diabetic nephropathy (DN). The objective of this study was to investigate genotypic and allelic frequencies of monocyte chemoattractant protein-1 (MCP-1) gene polymorphism in the healthy and patients with and without DN. The MCP-1 genotypes were determined in 43 patients with nephropathy and 43 without nephropathy and a control group of 105 healthy individuals. The genotype MCP-1 (−2518G/A) distribution did differ between the control group and the type 2 diabetic patients ($P = 0.004$). The frequency of the polymorphic G allele was also no similar for the group with type 2 diabetes as for the control group with 20.9 and 32.4%, respectively ($P = 0.012$). The AA genotype and A allele at MCP-1 −2518 was an independent risk factor for the progression of type 2 diabetes. In conclusion, MCP-1 AA genotype and A allele may play a specific role(s) in determining diabetic susceptibility, but do not seem to be important in the clinical manifestations of DN.

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Introduction

Type 2 diabetes is a well-recognized cause of chronic renal failure (CRF) [1]. Diabetic nephropathy (DN) eventually develops in ∼30% of patients with both type 1 and type 2 diabetes, and once present will progress in many of these individuals to end stage renal disease [2]. Hypertension, poor glycemic regulation, and albuminuria are recognized risk factors for the development or the progression of DN, apart from these factors could clarify all of the inter-individual variability's in the rate of progression to CRF [3].

However, as not all diabetic patients will develop DN, this support the hypothesis for factors of genetic susceptibility (or of protection!) to DN [4, 5].

Tissue macrophage accumulation is thought to induce insulin resistance during obesity and stimulate the progression of DN. Monocyte chemoattractant protein-1 (MCP-1) is a potent stimulator of macrophage recruitment. It is increased in adipose tissue during obesity and in diabetic kidneys, suggesting that inflammation of these tissues may be MCP-1 dependent [6]. MCP-1 is suggested to be implicated in the pathogenesis of DN by activating and recruiting monocytes to the glomerulus via regulation of adhesion molecule expressions [7]. Macrophages accumulate in diabetic kidneys in association with the local up-regulation of MCP-1 [8].

This study aims to investigate Turkish type 2 diabetic patients with/without DN and healthy group and examined the contribution of the MCP-1 (−2518G/A) gene polymorphism to the development of DN.

Subjects and methods

Subjects were selected from the Ege University Faculty of Medicine (Department of Endocrinology and Metabolism Diseases). Type 2 diabetes was diagnosed according to the criteria of the American Diabetes Association [9].

Diabetic nephropathy (microalbuminuria) was diagnosed when albumin excretion rate (AER), measured by radioimmunoassay (RIA), was 30–300 mg/24-h in at least two out of three 24-h urine collections over a 3-month period. At the beginning of the study, 50 diabetic patients with nephropathy and 50 diabetic patients without nephropathy has been taken to the study but 7 patients with nephropathy and 7 patients without nephropathy among those patients have been excluded from the study due to different reasons (personal reasons, drug usage history that leads to the nephropathy, reasons such as nephrolithiasis, amyloidosis etc.). In the study group, there are no patients with or without nephropathy that take ARB or ACE treatment. The subjects of this study were 86 diabetic outpatients (43 individuals who had type 2 diabetes mellitus with nephropathy (DN) and 43 individuals who just had type 2 diabetes mellitus without nephropathy), and the 105 individuals healthy control group. The control group consists of the persons to whom 75-g oral glucose test (0 and 2nd hours) was applied and found to be normal. Informed consent was obtained from all participants. A detailed medical history of each patient was obtained. Phenotypic characteristics were determined. Age, sex, blood pressure were recorded. Total cholesterol, triglyceride, serum creatinine, and urea levels were measured by the automated Olympus AU2700 Analyzer. HbA1c (%) and microalbuminurea levels were measured by the automated Chemistry Analyzer. A total of 191 individuals were screened for the presence of the MCP-1 (−2518G/A) gene polymorphism: 43 individuals who had type 2 diabetes mellitus and DN, 43 individuals who just had type 2 diabetes mellitus, and the remaining 105 individuals who constituted the healthy control group.

The −2518 MCP-1 genotyping

Genomic DNA was prepared from whole peripheral blood using Qiagen mini blood DNA purification kits (Qiagen, Mississauga, Ontario, Canada) according to manufacturer. A 930-base-pair (bp) segment of the MCP-1 59-flanking region between nucleotides −1817 and −2746 relative to the major transcriptional start site was analyzed. This segment contains the distal regulatory region of the MCP-1 gene and was shown to be important for cytokine induction of MCP-1 transcription. The distal regulatory region was PCR-amplified from genomic DNA using the forward primer 5′-CCGAGATGTTCCCAGCACAG-3′ and the reverse primer 5′-CTGCTTTGCTTGTGCCTCTT-3′ as follows: 1 μl (100 ng) of genomic DNA was added to amplification buffer containing 20 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each of dATP, 2′-deoxycytidine 5′-triphosphate, dGTP, 2′-deoxythymidine 5′-triphosphate, 10 pmol of each primer, and 1.0 U of Taq DNA polymerase (AmpliTaq, Perkin-Elmer, Norwalk, CT, USA, in total of 25 μl). The DNA was amplified by cycling at 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min (GeneAmp 9700). After 35 cycles, the reaction was extended for an additional 10 min at 72°C. The 930-bp PCR product was digested with *Pvu*II (MBI Fermentas, Lithuania) to identify the G/A polymorphism occurring in the last nucleotide of the restriction recognition site (CAGCTg). The −2518 (G/A) polymorphism affects *Pvu*II digestion of the MCP-1 distal regulatory region. *Pvu*II digests the 930-bp DNA segment from G/G homozygous individuals into 708- and 222-bp fragments. PCR products from A/A homozygous individuals do not cut with *Pvu*II.

Statistical analysis

SPSS 14.0 for windows (SPSS Inc., Chicago, USA) was used for statistical analysis of results. Distributions of continuous variables in groups were expressed as mean ± S.D. The distribution of alleles and genotypes between groups was compared using χ^2 analysis or Fisher's exact test. Differences between the mean of biochemical parameters were examined by means of ANOVA. A value of $P < 0.05$ was considered to be significant.

Results

Diastolic- and systolic-blood pressure, fasting plasma glucose, postprandial plasma glucose, HbA1c, total cholesterol, triglyceride, LDL-cholesterol, serum creatinine, serum urea, and microalbuminurea levels were significantly higher in type 2 diabetic patients either with or without DN than in the control group (Table 1).

Frequencies of MCP-1 (−2518G/A) gene polymorphism

We analyzed the frequency of MCP-1 (−2518G/A) gene in type 2 diabetes mellitus and control groups. The distributions of genotype and allele frequencies were compared between diabetic patients and controls (Table 2) as well as between patients with nephropathy and those without nephropathy (Table 3).

The genotype MCP-1 (−2518G/A) distribution followed relation between the control group (AA 46.7%, GA 41.9%, GG 11.4%) and the type 2 diabetes mellitus patients

Table 1 The clinical and metabolic characteristics of the diabetic patients and healthy control group

	With DN ($n = 43$) Mean ± SD	Without DN ($n = 43$) Mean ± SD	Control group ($n = 105$) Mean ± SD	P
Age (year)	53.38 ± 11.61	54.27 ± 10.50	53.26 ± 9.72	>0.05
Duration of diabetes (years)	8.87 ± 7.15	8.53 ± 5.19	–	>0.05
Systolic BP (mmHg)	128.43 ± 12.00	126.92 ± 14.00	109.36 ± 10.42	<0.05
Diastolic BP (mmHg)	80.56 ± 8.45	79.97 ± 7.16	70.32 ± 5.02	<0.05
Fasting plasma glucose (mg/dl)	124.72 ± 20.52	119.51 ± 24.12	83.77 ± 15.50	<0.05
Postprandial plasma glucose (mg/dl)	176.22 ± 23.40	175.97 ± 30.42	120.56 ± 8.62	<0.05
HbA1c (%)	7.1 ± 2.08	6.9 ± 2.31	5.10 ± 0.12	<0.05
Total cholesterol (mg/dl)	208.09 ± 28.71	207.93 ± 26.52	161.83 ± 41.50	<0.05
Triglyceride (mg/dl)	183.19 ± 18.02	178.58 ± 23.16	125.25 ± 97.92	<0.05
HDL-cholesterol (mg/dl)	40.37 ± 10.15	41.98 ± 10.41	55.77 ± 12.17	<0.05
LDL-cholesterol (mg/dl)	142.15 ± 27.36	139.55 ± 21.68	103.84 ± 23.57	<0.05
Serum creatinine (mg/dl)	1.00 ± 0.11	0.97 ± 0.12	0.6 ± 1.1	<0.05
Serum urea (mg/dl)	46.08 ± 6.00	45.82 ± 8.27	24.52 ± 6.92	<0.05
Microalbuminuria (mg/day)	204.19 ± 23.85	21.82 ± 4.33	10.07 ± 3.40	<0.05

n = Number of individuals

If $P < 0.05$ in chi-square test, it is accepted as statistically significant

Table 2 Genotype and allele frequencies of the monocyte chemoattractant protein 1 (MCP-1) 2518G/A gene polymorphism in with diabetic patients and healthy controls

MCP-1 gene	Patients ($n = 86$)	Control groups ($n = 105$)
Genotypes		
AA	50 (58.1%)	49 (46.7%)
GA	36 (41.9%)	44 (41.9%)
GG	0 (0%)*	12 (11.4%)
Alleles		
A	136 (79.1%)*	142 (67.6%)*
G	36 (20.9%)	68 (32.4%)

n, Number of patients who could be genotyped successfully

If $P < 0.05$ in chi-square test, it is accepted as statistically significant (* $P < 0.05$)

Table 3 Genotype and allele frequencies of the MCP-1 (2518A/G) gene polymorphism in type 2 diabetic patients with and without DN

MCP-1 gene	Diabetic nephropathy ($n = 43$)	Without diabetic nephropathy ($n = 43$)
Genotypes		
AA	24 (55.8%)	26 (60.5%)
GA	19 (44.2%)	17 (39.5%)
Alleles		
A	67 (77.9%)	69 (80.2%)
G	19 (22.1%)	17 (19.8%)

n, Number of patients who could be genotyped successfully

If $P < 0.05$ in chi-square test, it is accepted as statistically significant

(AA 58.1%, GA 41.9%, GG 0%). The frequency of the polymorphic MCP-1 GG genotype was significantly lower in the type 2 diabetes mellitus compare to control group with 20.9% and 32.4%, respectively ($P = 0.004$) (Table 2). MCP-1 gene C allele was statistical significantly higher than G allele in patient and control groups ($P = 0.008$).

Genotype MCP-1 (−2518G/A) frequencies in diabetic patients with nephropathy were (A/A) 55.8%, (G/A) 44.2%; (G/G) 0% versus (A/A) 60.5%; (G/A) 39.5%; (G/G) 0% in those without nephropathy (Table 3). Genotype MCP-1 (−2518G/A) frequencies were not statistical significant in diabetic patients with nephropathy compare to without nephropathy patients ($P = 0.414$). The frequency of the polymorphic G allele statistical non-significant higher was 22.1% in diabetic patients with nephropathy versus 19.8% in those without nephropathy ($P > 0.05$) (Table 3).

Discussion

Poor glycemic control, hypertension, and duration of diabetes are risk factors for the development of DN. Genetic susceptibility plays an important role in the pathogenesis of DN [10]. Glomerular macrophage accumulation in diabetes implicates monocyte recruitment mechanisms in the pathogenesis of DN [11]. Chemokines play a prominent role in the acute inflammatory response in several models of kidney disease [12]. In a large cohort of Caucasians, the MCP-1 G-2518 gene variant was significantly and negatively correlated with plasma MCP-1 levels and the prevalence of insulin resistance and type 2 diabetes [13].

Zietz et al. investigated the association of serum levels and the −2518 A → G promoter polymorphism of the gene for chemokine MCP-1, a major chemoattractant of monocytes and activated lymphocytes, with metabolic parameters as well as insulin, leptin, and the cytokines tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) in 534 Caucasian patients with type 2 diabetes mellitus. In this study, compared to the literature, MCP-1 levels were found to be substantially higher in patients with type 2 diabetes mellitus. In contrast, genotype frequencies were similar compared to those in non-diabetic patients and were not related to MCP-1 levels [14]. Mori et al. investigated 277 Japanese patients diagnosed with IgA nephropathy (IgAN) based on renal biopsy to clarify the association between the progression of IgAN and MCP-1 gene polymorphism at position A-2518G, which regulates the transcription of the MCP-1 gene. The AA genotype at MCP-1 −2518 was an independent risk factor for the progression of renal disease in Japanese patients with IgA nephropathy, and was closely associated with renal survival [15]. Tucci et al. examined the role of a functional MCP-1 polymorphism in systemic lupus erythematosus (SLE) and lupus nephritis (LN). These results suggest that an A/G or G/G genotype may predispose to the development of SLE and further indicate that SLE patients with these genotypes may be at higher risk of developing LN [16].

In our study, we aimed to evaluate the relation between the genotypic and allelic frequencies of the MCP-1 −2518 A → G promoter polymorphisms, and their association with the risk to develop DN in Turkish population.

Some investigators reported G at −2518 more MCP-1 production and were a risk factor for kidney allograft failure in patients who underwent kidney transplantation [17, 18]. However, in our study, the polymorphisms of MCP-1 GG genotype ($P = 0.004$) frequencies were significantly lower with the occurrence of type 2 diabetic patients.

In a Korean study, was described A allele carriage significantly relation with progressive kidney failure in Korean patients with type 2 DM [19]. In our study, the MCP-1 −2518 A → G genotype and allele frequencies were not different between diabetic patients with nephropathy and without nephropathy ($P = 0.414$). This study is the first one in Turkish population which shows that MCP-1 −2518 A → G gene polymorphism was not associated with risk of the development of nephropathy.

As a result, our study suggests that MCP-1 −2518 A → G gene polymorphism may not play a specific role(s) in determining DN susceptibility. We think that this discrepancy may be due to the difference in either the degree of the nephropathy or the duration of diabetes. Limited number of patients should contribute the negative results of our study. However, in this study, we found GG genotype was statistical significant lower than control subjects in Turkish patients with type 2 DM.

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