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Effect of G(-174)C polymorphism in interleukin-6 gene on cardiovascular disease in type 2 diabetes patients

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ABSTRACT

Interleukin-6 (IL-6) is an important pro-inflammatory cytokine of relevance to cardiovascular diseases. The aim of this case-control study was to evaluate the association between the G(-174)C functional polymorphism in the IL-6 gene and risk of cardiovascular disease (CVD) in type 2 diabetes patients. We examined 1090 patients with T2DM and 612 controls. All subjects were genotyped for the G(-174)C polymorphism by polymerase chain reaction (PCR) and restriction analysis. There were no significant differences in the distribution of genotypes and alleles between T2DM patients and healthy controls. Significantly higher C allele frequency was observed in CVD+ patients compared to CVD- subgroup (53% vs. 32%, $p < 0.0001$). The odds ratio for C allele was 2.4 (95% CI 1.99–2.9, $p < 0.0001$) and for CC genotype 4.55 (95% CI 3.12–6.63, $p < 0.000$). When the distribution of G(-174)C polymorphism was compared in subgroups with different clinical phenotypes of CVD, a significant association of CC genotype with myocardial infarction was observed. Forty eight percent of patients with MI had the CC genotype compared to 22% of patients without MI ($p < 0.0001$). In conclusion, type 2 diabetes patients carrying the C allele of the IL-6 G(-174)C polymorphism have a significantly increased risk of CVD.

1. Introduction

Diabetes mellitus (DM), a complex metabolic disease, increases the risk for macrovascular complications that are associated with cardiovascular diseases, mainly atherosclerosis, coronary artery disease, hypertension and stroke [1,2]. The increased evidence of these complications in diabetic population is thought to be due to several pathological factors such as hyperglycemia, hyperlipidemia, growth factors, inflammatory cytokines and chemokines, and advanced glycation end products [3,4].

Chronic inflammation is recognized as a central component of atherosclerosis. Genes encoding inflammatory cytokines might thus predispose to cardiovascular diseases. Interleukin-6 (IL-6) has both pro- and anti-inflammatory effects. It is secreted by several tissues and cell types, like monocytes and macrophages, lymphocytes T and B, adipocytes, fibroblasts and mesangial and endothelial cells [5,6]. IL-6 regulates various pathophysiological processes, including acute phase response, inflammation, immune response, host defense mechanisms, hematopoiesis and cellular growth [7]. Circulating levels of IL-6 are genetically regulated. The IL-6 gene maps to chromosome 7p21. It contains 7 exons, covering approximately 12.8 kb of genomic DNA [8]. A single nucleotide polymorphism, G174C in the promoter region of the IL-6 gene, influences the level of IL-6 expression [9]. Several studies reported its association with inflammatory and autoimmune disorders [10]. It is also involved in atherosclerosis and cardiovascular events [11–13]. IL-6 contributes to cardiovascular disorders by affecting metabolic, endothelial and coagulant events [14,15]. Interleukin-6 is strongly associated with markers of inflammation, such as C-reactive protein (CRP). It may be a potential link between risk factors and biological mechanisms for cardiovascular disease [16].

The aim of the present study was to evaluate the effect of G(-174)C polymorphism (rs1800795) in the IL-6 gene on cardiovascular disease in type 2 diabetic patients.

Abbreviations: IL-6, interleukin-6; T2DM, type 2 diabetes mellitus; CVD, cardiovascular disease; MI, myocardial infarction; PCR, polymerase chain reaction; CRP, C-reactive protein.

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2. Materials and methods

2.1. Study subjects

The study population included 1090 unrelated T2DM patients, consecutively enrolled between 2008 and 2012 from the Departments of Nephrology and Cardiology of Medical University of Lublin. All subjects were Caucasians of Polish origin. The type 2 diabetes was diagnosed according to American Diabetes Association criteria [17]. Diagnosis was based on one or more of the following conditions: classic symptoms of hyperglycemia (polyuria, polydipsia, weight loss), random plasma glucose ⩾ 11 mmol/L or fasting plasma glucose ⩾ 7 mmol/L and/or treatment with antidiabetic agents. Age at diagnosis of T2DM was >35 years in all subjects. The mean duration of diabetes was 13.7 years (range 7–31). Cardiovascular disease was diagnosed in 738 patients (68%) as one or combination of several pathological states: ischemic heart disease (chronic coronary heart disease and/or acute coronary syndromes), congestive heart failure, ischemic cerebral stroke, or peripheral arteriopathy. Clinical manifestations of CVD were confirmed/excluded by relevant biochemical, radiographic, echocardiographic and vascular diagnostic criteria. Among the patients 722 individuals (66%) were hypertensive, according to World Health Organization criteria. Patients with hypertension had persistent systolic blood pressure > 140 mm Hg and diastolic blood pressure > 90 mm Hg and/or were currently treated for hypertension. Healthy control subjects of Polish origin ($n = 612$) were unrelated volunteers (mainly blood donors and hospital staff members) without history of diabetes or cardiovascular events and no signs of dysfunction of cardiovascular system upon examination. The exclusion criteria included also abnormal lipid profile, obesity and hypertension. Subjects with positive family history of DM or CVD in first degree relatives were excluded. A written informed consent for genotyping was obtained from all patients and controls in accordance with principles of the 1964 Declaration of Helsinki. The institutional ethics committee approved the protocol of the study.

2.2. Determination of IL-6 G(-174)C genotype

Genomic DNA was extracted from peripheral blood leukocytes obtained by the standard procedure and stored at −20 °C until use. The G(-174)C polymorphism in the IL-6 gene was analyzed by amplification of 165 bp DNA fragment by polymerase chain reaction (PCR) with the following primers used for amplification: sense primer 5′-AGGAAGAGTGGTTCTGCTTC-3′ and antisense primer 5′-CTTTGTTG GAGGGTGAGGGTG-3′. The reactions were carried out in 96-well microtiter plates in GeneAmp® PCR System 9700 (Applied Biosystems). Genomic DNA (300 ng) was amplified in 30 μl reaction using the following conditions: initial denaturation at 95 °C for 6 min, followed by 30 cycles of 94 °C for 45 s, annealing at 60 °C for 45 s and extension at 72 °C for 1 min. A final extension step at 72 °C for 10 min completed the reaction. Ten μl of the PCR product were digested overnight at 37 °C with 5 U of Nla III restriction endonuclease (Thermo Scientific, Waltham, MA) and resulting fragments were separated on a 2% agarose gel. Observed fragment sizes were 81 bp + 77 bp for the C allele and 165 bp for the G allele. The quality of genotyping was tested by using blind DNA duplicates for random samples (10% of all samples). In addition, for each genotype 20 samples were randomly selected and PCR products were sequenced in CEQ 8000 Genetic Analysis System (Beckman Coulter, England). There was a 100% concordance between genotyping assays.

2.3. Statistical analysis

Statistical calculations were performed using SPSS software, version 11.0 for Windows (SPSS, Inc., Chicago, IL). The normally distributed continuous variables for baseline characteristics are presented as percentages or means ± SD. The Hardy–Weinberg equilibrium was assessed using a χ^2 test with 1 degree of freedom. Distribution of genotypes and alleles was compared between groups using a Pearson χ^2 test of independence with 2×2 contingency and z statistics (for genotype counts < 5, Fisher test was used). Pearson χ^2 and Mann–Whitney tests were used for comparing discrete and continuous variables. For significant associations the adjusted odds ratios (OR) with 95% confidence intervals (CI) were calculated using an unconditional logistic regression model. Power calculations were performed with the program of Purcell et al. [18] (available at http://pngu.mgh.harvard.edu/~purcell/gpc/). The sample size in both cohorts including 1090 T2DM patients and 612 healthy subjects was adequate to give the power of 80% (probability of not occurring a type II error). Multiple logistic regression analysis was performed to determine the relationship between risk factors, genotype and cardiovascular disease. P values < 0.05 (two-tailed) were considered statistically significant.

3. Results

The G(-174)C polymorphism in the IL-6 gene was analyzed in 1090 patients with T2DM and 612 healthy control subjects. In the patient group 738 subjects (68%) had CVD. The demographic and clinical characteristics of T2DM subjects with and without cardiovascular disease are summarized in Table 1. T2DM patients with CVD were on average 11 years older than those without CVD. The male/female ratio was comparable in both CVD+ and CVD- subgroups ($p = 0.315$). The CVD+ patient subgroup, as expected, showed a higher prevalence of conventional risk factors for CVD, including higher levels of total cholesterol and triglycerides ($p = 0.013$ and $p < 0.01$, respectively) and lower levels of HDL cholesterol ($p = 0.025$). BMI in the CVD+ subgroup was higher than in CVD− subjects ($p < 0.05$).

The genotype distribution and allele frequencies of G(-174)C SNP in healthy subjects were similar to those reported for Polish and other European populations [19–21]. The prevalence of IL-6 G(-174)C genotype and allele frequencies in T2DM patients and controls is presented in Table 2. The distribution of IL-6 genotypes in both groups was in Hardy–Weinberg equilibrium ($p = 0.878$ and 0.497, respectively). No significant differences in either genotype or allele frequencies of IL-6 polymorphism were observed between healthy controls and patients with T2DM. The OR for the presence of minor C allele was 1.08 (95% CI 0.94–1.24, $p = 0.284$).

Table 1
Main demographic and clinical characteristics of T2DM subjects with and without cardiovascular disease.

Variable	T2DM CVD+ ($n = 738$)	T2DM CVD− ($n = 352$)	p value[a]
Male/female	376/362	162/190	0.315
Age at study (years)	65.8 ± 16	54.3 ± 14.6	0.000
Diabetes duration (years)	14.8 ± 7.2	12.7 ± 4.8	0.000
Cardiovascular disease (%)	738 (100)	0 (0)	0.000
Microvascular complications (%)	693 (94)	186 (53)	0.000
Essential hypertension (%)	598 (81)	225 (64)	0.0004
HbA_{1c} (%)	7.9 ± 2.6	7.7 ± 2.4	0.345
Total cholesterol (mmol/l)	4.9 ± 1.3	4.7 ± 1.3	0.013
HDL cholesterol (mmol/l)	1.2 ± 0.6	1.5 ± 0.6	0.025
Triglycerides (mmol/l)	2.1 ± 1.2	1.7 ± 0.6	<0.01
Body mass index (kg/m^2)	28.1 ± 4.5	26.8 ± 5.1	0.026

T2DM, type 2 diabetes mellitus. CVD, cardiovascular disease. Values are means ± SD or numbers (%) as appropriate.
[a] Pearson's χ^2 test used for categorical variables, Mann–Whitney test for continuous variables.