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Association of Matrix Metalloproteinase 3 and 11β-Hydroxysteroid Dehydrogenase Type 1 Gene Polymorphisms with Type 2 Diabetes in Koreans

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ABSTRACT

Type 2 diabetes is a typical polygenic disease complex, for which several common risk alleles have been identified. Matrix metalloproteinase-3 (MMP3) is an enzyme known to be involved in the destruction of the extracellular matrix in normal physiological processes and disease. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) that catalyzes the conversion of inactive cortisone to active cortisol. is related to the development of type 2 diabetes. Therefore, we investigated the genotypes for the SNP rs522616 in MMP3 gene and rs12086634 in 11β-HSD1 gene between patients and the control group in Korean population. One hundred patients (Male 58, Female 42) with type 2 diabetes (T2D) and 100 controls (Male 36, Female 64) participated in this study. As a result, there was no association between the SNP rs522616 in MMP3 gene, rs12086634 in 11β-HSD1 gene and T2D. Further studies with larger population may be needed for the development of diagnostic methods at the genetic level.

Key words : 11β-Hydroxysteroid dehyrogenase, Matrix metalloproteinase 3, Type 2 diabetes, Polymorphism, Metabolic syndrome

Introduction

Type 2 diabetes (T2D) is characterized by insulin resistance and relative insulin deficiency [1,2]. T2D is a typical polygenic disease complex, and several single nucleotide polymorphisms (SNPs) have been associated with risk or protection regarding this disease and its complications [3-5].

Among candidate genes, a common polymorphism in the promoter of Matrix metalloproteinase 3 gene (MMP3, also known as stromelysin 1) has been reported to be associated with type 2 diabetes [6]. Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes [7]. A recent study suggested serum concentrations of matrix metalloproteinase (MMP) 3 and MMP9 were evaluated in 82 asymptomatic type 2 diabetes mellitus patients without cardiovascular complications and in 41 non-diabetic control subjects [6]. Matrix metalloproteinases (MMPs) include most major constituents of the arterial wall as substrates [8,9]. Changes in the expression of MMPs in vascular and cardiac tissues have been implicated in the pathogenesis of several cardiovascular conditions including atherosclerosis, vascular calcification [10].

MMP3 is expressed in human coronary atherosclerotic lesions and is known to be involved in degradation of the plaque and to be co-localized with calcium and fibrin deposits in advanced lesions, indicating a possible role of MMP3 in arterial calcification [11,12]. The MMP3 gene promoter polymorphism

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is associated with increased coronary artery calcification [13]. A cluster of nine additional MMP genes (MMP7, MMP20, MMP27, MMP8, MMP10, MMP1, MMP3, MMP12, and MMP13) maps within a 500 kb region located at chromosome 11q22. The MMP3 entire gene was sequenced (2,900 bp upstream of the transcription start site, all 10 exons and 9 introns, and 2,400 bp of the 3′-UTR) [14]. The rs522616 (−709A>G) polymorphism is located in the 5′-UTR-709 bases upstream of the transcription start site [15].

Polymorphoisms of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) were reported to be associated with type 2 diabetes and insulin resistance in Pima Indians [16]. 11β-HSD1, converts inactive glucocorticoids (GCs) into active GCs plays a role in the pathogenesis of insulin resistance [17]. 11β-HSD1 overexpression in adipose tissue generates visceral obesity and systemic insulin resistance in transgenic mice. Liver-specific overexpression of 11β-HSD1 leads to insulin resistance in the absence of obesity in mice [18]. 11β-HSD1 elevation in adipocytes is proposed to be a common molecular etiology for visceral obesity and metabolic syndrome [19]. The encoding gene, 11β-HSD1 is located close to the end of the long arm of chromosome 1 in mice and humans and chromosome 13 in rats [20]. 11β-HSD1 mRNA and activity are present in mouse and human pancreatic islets [21]. 11β-HSD1 activity is increased in islets from diabetic rodents [22].

We investigated whether MMP3 (rs522616), 11β-HSD1 (rs12086634) gene polymorphisms is associated with T2D in Korean population. Therefore, the aim of this study was to analyze the MMP3 and 11β-HSD1 polymorphisms in Korean subjects, and the possible association of the MMP3 and 11β-HSD1 polymorphisms with T2D. We hypothesized the polymorphism of MMP3 and 11β-HSD1 gene can be associated with T2D.

Materials and Methods

1. Study subjects

This study included 200 subjects consisting of 100 patients with Type 2 diabetes mellitus and 100 healthy controls in Korea. Type 2 diabetes mellitus group was selected among persons who visited Kyungpook National University. All participants gave their written informed consent.

Diabetic subjects were defined as follows 1) if they had history of type 2 diabetes, or 2) if serum levels of fasting glucose were 126 mg/dL or higher, or 3) serum levels of 2 hours postprandial glucose were 200 mg/dL or highthey were regarded as type 2 diabetes. Control group were defined as follows 1) fasting glucose was 100 mg/dL or lower, and 2) Hb A1c (glycosylated hemoglobin A1c) level was 5.8% or lower, and 3) age was older than 60-years-old.

The subjects underwent a standardized evaluation consisting of a questionnaire, physical examination, and laboratory tests. Weight (without shoes, light outdoor clothing) and height were measured by trained personnel and body mass index (BMI, kg/m^2) was calculated. Waist circumference was measured at the narrowest point as viewed from the front.

2. Blood sampling and DNA extraction

Blood samples were obtained each subject by venopuncture. Genomic DNA was extracted from the ethylenediaminetetraacetic acid (EDTA) anticoagulated blood with a standard phenol/chloroform procedure. The Puregene DNA Isolation Kit was used according to the manufacturer's : A 3 mL RBC lysis solution was added in 1 mL whole blood and then was inverted to mix and incubated 5 minutes at room temperature. After centrifuging for 5 minutes at 4˚C at 3500 rpm, was poured off supernatant and vortexed to resuspended the cell pellet. There was dispensed 1 mL cell lysis solution in to each tube of resuspended cells, vortexed for 10 seconds and then was added 0.5 mL protein precipitation solution and was vortexed for 10 seconds. After centrifuging for 10 minutes at 4˚C at 3500 rpm, was poured supernatant and vortexed to resuspended the cell pellet. There was added 1.5 mL isopropyl alcohol to the supernatant containing the DNA and mixed the samples carefully by inverting until the white DNA thread appear. After centrifuging for 5 minutes at 2500 rpm, was poured off supernatant. The pellet was washed with 70% ethanol and repelleted, and the ethanol was carefully drained and the pellet was dried. 50 μL of DNA rehydration solution was added to the pellet.

3. Genotyping of sequence variations in MMP3 and 11β-HSD1

We genotyped the MMP3 polymorphism (rs522616) and 11β-HSD1 (rs12086634) in 100 patients with Type 2 diabetes mellitus and 100 healthy controls by real-time polymerase chain reaction (PCR) analysis using a Light cycler™ (Roche Diagnostics, Germany). All the primers and probes were designed appropriately with Light Cycler Probe Design2 soft-

ware (Roche). Probes and forward and reverse primers were prepared for each reaction.

4. Statistical analysis

Statistical analyses were performed using the SPSS (version 15.0). Continuous data were reported as means±standard deviation. Deviations from Hardy-Weinberg equilibrium were tested using a χ chi-square test. We compared the distribution of genotype between type 2 diabetes and control groups by chi-square tests. Multiple logistic regression analysis was performed on both groups. The Odd ratio (OR) and 95% confidential interval (CI) were also calculated. OR was calculated by adjustment of age, sex, BMI (kg/m^2), smoking status (nonsmoker, ex-smoker and current smoker) and drinking status (nondrinker, ex-drinker and current drinker). P value <0.05 (two-sided) was considered as significant. P value were not adjusted for multiple testing.

Results

1. Baseline characteristics of control and type 2 diabetes groups

We analyzed T2D and control groups. The clinical characteristics of each group are presented in Table 1. There were significant differences in the distributions of age between the T2D and control groups. Total cholesterol ($P<0.001$), High-density lipoprotein ($P=0.039$), Low-density lipoprotein ($P<0.001$), Triglyceride ($P<0.001$) levels and HbA1c ($P<0.001$) were differed significantly between the two groups. There were no differences in clinical characteristics, such as gender distribution, BMI, smoking status, between T2D and control groups (Table 2).

2. Association of polymorphisms with type 2 diabetes

All genotype frequencies in each group were in Hardy-Weinberg equillibrium ($P<0.05$). The SNP rs522616 in MMP3 and rs12086634 in 11β-HSD1 gene showed no significant association with T2DM. There was no difference in the SNP allele frequency between the control and type 2 diabetes groups. In subgoup analysis by gender, there was also no significant association between the polymorphism studied and T2DM (data not shown).

Table 1. Dermographic and health behavior variables among study subjects

Variables	Controls (n=100)	Cases (n=100)
Age (year)	63.24±2.79	64.62±2.81
BMI[a] (kg/m^2)	23.51±2.89	28.13±4.32
Sex		
Male	36	58
Female	64	42
Total cholesterol (mmol/L)	192.21±32.88	177.44±34.33
HDL-cholesterol[b] (mmol/L)	46.76±11.37	42.92±6.73
LDL-cholesterol[c] (mmol/L)	123.52±29.27	132.15±21.82
Triglyceride (mmol/L)	109.66±48.07	142.64±41.27
FBS (mmol/L)	89.09±5.89	123.38±48.02
Smoking (%)	20	51.4
HbA1C	5.31±0.31	6.88±2.11
hsCRP[d]	1.55±3.13	0.65±3.12

Data are shown as means±the standard deviation. [a]Body mass index, [b]High density lipoprotein cholesterol, [c]Low density lipoprotein cholesterol, [d]High-sensitivity C-reactive protein.

Table 2. HSD11B1 and H6PD4 genotypes in the control and T2D group

Genotype	Controls (%)	Cases (%)	P
rs522616			0.286
AA	93	88	
AG	7	10	
GG	0	2	
rs12086634			0.352
TT	26	28	
GT	52	55	
GG	22	17	

Genotype distributions are shown as number (%). Values are means±SD (%). p-Values were adjusted for age, sex. T2D, type 2 diabetes; n, number

Discussion

This study showed no significant association betweend SNP rs522616 in MMP3 gene, rs12086634 in 11β-HSD1 gene and type 2 diabetes in Korean population. This significance was maintained after adjusting for the age and gender. However, after considering multiple comparisons, this significance was lost.

There are a few studies concerning the association of the MMP gene polymorphism with type 2 diabetes [6,14]. In asymptomatic type 2 diabetic patients without cardiovascular complications, serum MMP3 and MMP9 were elevated, MMP9 was associated with HbA (1c) and MMP3 was associated with albuminuria [6]. Also, significant differences between cases and controls were observed for MMP3 genotype frequencies ($P<0.01$). The 6A allele was high frequently seen in the dis-

ease group, compared with the control group in the Iranian T2DM studied [14].

Another studies investigated the association of 11β-HSD1 SNPs with Type 2 diabetes, plasma insulin concentrations and insulin action, independent of obesity in Pima Indians and 11β-HSD1 in adipose tissue and prospective changes in body weight and insulin resistance [16,23].

In conclusion, the rs522616 polymorphism of the MMP3 gene and the rs12086634 polymorphism of the 11β-HSD1 gene were not associated with T2D in Korean population. Further studies with larger sample sizes are required for association between MMP3 and 11β-HSD1 polymorphism and the pathogenesis of insulin resistance, T2DM in the Korean population. If our findings are confirmed in a large sample and in other populations, the MMP3 and 11β-HSD1 polymorphism may provide an important genetic marker to identify people at risk of T2D and to predict people at risk of cardiovascular complication of T2D for treatment and prevention of T2D.

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