The C-174G promoter polymorphism of the *interleukin-6 (IL-6)* gene that affects insulin sensitivity in Caucasians is not involved in the pathogenesis of Taiwanese type 2 diabetes mellitus

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ABSTRACT. Interleukin-6 (IL-6) is a pleiotropic cytokine which regulates the immune response, the acute-phase response, hematopoiesis and body energy balance. Genetic polymorphism at -174 position of *IL-6* promoter has been recently reported to be linked with insulin resistance, however, with conflicting results. The C allele at *IL-6* -174 position is associated with increased insulin sensitivity, and has a protective role for the development of type 2 diabetes, in a Spanish study. Whereas, according to a Finnish study, it is correlated with lower insulin sensitivity and may encourage the development of type 2 diabetes. Ethnic differences play certain roles in the distribution of *IL-6* promoter polymorphisms because the distribution of the *IL-6* -174 C allele is diverse among study subjects with different racial origins. Therefore, we examined *IL-6* C-174G polymorphism in Taiwanese type 2 diabetic subjects to clarify the relationship of this polymorphism with Taiwanese type 2 diabetes mellitus in the context of the aforementioned mentioned contradictory results. All of our 101, type 2 diabetic patients and 112, non-diabetic, healthy individuals carried homologous G alleles. No C allele was found. Our study suggested that the C allele at the *IL-6* -174 position was rare in Taiwanese people. Furthermore, our results demonstrated that *IL-6* C-174G polymorphism is unlikely to play a role in the development of Taiwanese type 2 diabetes, regardless of its protective or promoting role.

Keywords: *IL-6* C-174G promoter polymorphism, type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disease characterized by insulin resistance and impaired pancreatic islet β-cell function. In 1997, Pickup *et al.* proved the hypothesis that T2DM is a manifestation of an acute-phase response, by showing that acute-phase/stress reactants (serum sialic acid, C-reactive protein, etc), and the major cytokine of the acute-phase response, interleukin-6 (IL-6), were elevated in patients with T2DM, particularly in those with features of the insulin resistance syndrome [1]. The authors suggested that elevated IL-6 levels can stimulate hepatic acute-phase protein production and contribute to diabetogenic mechanisms. Therefore, the acute phase response may be one mechanism to explain many of the clinical and biochemical characteristics, such as the increased secretion of very low-density lipoprotein (VLDL), the reduced serum high-density lipoprotein and dyslipidaemia, of T2DM. Evidence regarding the correlation of the acute-phase response, IL-6 and T2DM has emerged thereafter.

IL-6 is a multifunctional cytokine which is expressed in adipose tissue, skeletal muscle and the hypothalamus. Plasma IL-6 is significantly increased in type 2 diabetes, compared to that of normal subjects, and is significantly associated with body mass index (BMI) in diabetic patients [2]. However, basal production of IL-6 in cultured diabetic blood cells is markedly depressed in comparison with that of non-diabetic samples. The decreased IL-6 basal production capacity is related to a high triglyceride concentration. Eggesbø *et al.* [3] also observed an inverse correlation between VLDL and lipopolysaccharide-stimulated IL-6 production from peripheral blood mononuclear cells. A recent report showed that adipose tissue can secrete IL-6, and this release is greater in obese subjects. Furthermore, IL-6, as well as glucose and insulin levels, increases postprandially in the interstitial fluid of subcutaneous adipose tissue [4]. These observations suggest that IL-6 might modulate adipose glucose and lipid metabolism.

The gene encoding IL-6 is located on chromosome 7. The C-174G polymorphism of the 5' promoter region in *IL-6*

can regulate transcription activity of the *IL-6* gene in homozygous subjects carrying the C allele at this position, because the C allele is associated with lower plasma IL-6 levels. The polymorphism of the *IL-6* gene at position -174 is associated with insulin resistance, postload glucose levels, and two markers of putative IL-6 action: peripheral white blood cell (WBC) count and glycosylated cortisol binding globulin. Recently, a Spanish study demonstrated that homozygous individuals carrying the C allele (11 out of 32, 34.4%) at position -174 of the *IL-6* gene have a significantly lower integrated area-under-the-curve of serum glucose concentrations after oral glucose tolerance test, lower blood glycosylated hemoglobin, fasting insulin levels, total and differential WBC count, and an increased insulin sensitivity index than carriers of the G allele of similar age and body composition [5]. A study by Vozarova *et al.* [6] also demonstrated that the GG genotype was associated with T2DM in Spanish Caucasian subjects and American Indian subjects with non-Pima admixture. The above results demonstrated that individuals carrying the G allele would be more susceptible to developing insulin resistance, increased plasma markers of the acute-phase response and, subsequently, T2DM. However, contradictory results exist. The C-174G promoter polymorphism of the *IL-6* gene was reported to affect energy expenditure and insulin sensitivity in healthy, Finnish, normoglycemic subjects [7]. Subjects with the homologous *IL-6* -174 CC genotype had lower insulin sensitivity, lower rates of glucose oxidation and nonoxidative glucose disposal, compared with the subjects with the heterologous CG and homologous GG genotypes [7]. The authors claimed that their results are more convincing than the observations from the Spanish study mentioned above because they studied a relatively larger number of subjects (n = 124 v.s n = 32), and measured insulin sensitivity with the gold standard hyperinsulinemic-euglycemic clamp test.

Interethnic variation is identified in the frequencies of *IL-6* genetic polymorphisms. The prevalence of the *IL-6* -174 C allele is reported to range from 4.45% in Afro-Caribbeans [8], 13.85% in Gujarati Indians [8], 40 ~ 50% in Caucasians [9] to 62% in Spanish Caucasians [10]. In the context of the inconsistent correlation between *IL-6* C-174G genetic polymorphism and insulin sensitivity observed from different populations as mentioned earlier, as well as the differential distribution of *IL-6* C-174G polymorphisms among study subjects with different ethnic origins, our present study aimed at the investigation of this polymorphism in Taiwanese T2DM patients to clarify the correlation of this polymorphism with T2DM.

DONORS AND METHODS

Study subjects

Genomic DNA was extracted from fasting venous blood of 101, type 2 diabetic patients and 112, non-diabetic control subjects from the diabetic clinic in the Department of Internal Medicine, Chung Shan Medical University-Hospital. The diagnosis of T2DM was based on the clinical characteristics, magnitude of residual insulin or C-peptide secretory responses. For reference, 112, healthy, nondiabetic individuals were recruited by selecting those with normal blood glucose, C-peptide, and insulin during the 3 h after 500 kcal of food intake (meal test), and with no diabetic family history. Written consent were obtained from all the study subjects after the nature of the procedure was explained. The information regarding body height, weight, BMI, age, fasting blood sugar, renal function index (creatinine and blood urea nitrogen), etc. was also collected for further statistical analysis. The study was reviewed and approved by the institution's ethics committee.

Analysis of the C-174G polymorphism in the IL-6 gene

An aliquot of the DNA (50-100 *n*g) was used for amplification of the C-174G polymorphism using polymerase chain reaction (PCR). Briefly, primers flanking a 243 base-pair region spanning the C-174G polymorphism of the *IL-6* gene (IL-6F: 5'-TTGTCAAGACATGCCAAGTGGT-3' and IL-6R: 5'-GGGAAAATCCCACATTTGATAA-3') were used in PCR reaction. The DNA amplification was performed in a 20 mL volume containing 10 *p*mol of each primer, 4.5 mM $MgCl_2$, 0.25 mM of each dNTP, 1U Taq polymerase, and 1.5 mM super Taq buffer. Samples were amplified using the following conditions: an initial incubation of 95 °C for 5 min, then 30 cycles of 95 °C 1 min, 55 °C 1 min, 72 °C 1 min, followed by a final 5 min extension time. The amplified products were digested with *Nla* III to detect either the G or C allele. The reaction was stopped by addition of 5 mL agarose gel loading buffer and the results were checked by polyarcylamide gel electrophoresis.

RESULTS AND DISCUSSION

We examined the *IL-6* gene C-174G polymorphisms in 112, healthy Taiwanese control individuals (57 male and 55 female, with an average age of 48.2 years, BMI 24.98, fasting glucose 93.85 mg/dL, and WBC count [6,531 ± 159 (mean ± standard error)] × 10^9/mL), and 101 patients with type 2 diabetes (56 male and 45 female, with an average age of 57.1 years, BMI 24.66, fasting glucose 170.61 mg/dL, and WBC count [8,027 ± 427] × 10^9/mL) recruited from the diabetic clinic in the Department of Internal Medicine, Chung Shan Medical University-Hospital. The *IL-6* C-174G polymorphisms were detected by PCR-restriction fragment length polymorphism as described in Methods.

Our results showed that all the subjects in this study, including healthy and type 2 diabetic individuals, carried homologous G alleles at -174 position of *IL-6* promoter. No *C* allele was found. This observation demonstrated the G to C polymorphism at -174 of the *IL-6* gene was rare in Taiwanese people. Our results indicated that the previous observation that *IL-6* C-174G polymorphism may play some role in the pathogenesis of T2DM and insulin resistance, as well as lipid metabolism in Caucasians, was unlikely to be relevant in Taiwanese patients, regardless of the suggested protective [5] or promoting [7] role of C allele at this polymorphism. Notably, the prevalence of T2DM varies from population to population, with the highest rate found in Pima Indians (as high as ~ 50%). According to the observation that Spanish Caucasian individuals and American Indian subjects with non-Pima admixture with *IL-6* -174 G alleles are prone to type 2 diabetes [5, 6]; the frequency of T2DM in the Taiwanese population, in which the "protective" C allele is quite rare, should be higher than that in the Caucasian population

with its much higher prevalence of the "protective" C allele. However, it is intriguing that the prevalence of T2DM in the Taiwanese (about 1.5%) population is much lower than that in Caucasians (4 ~ 16%). Conversely, the incidence of type 2 diabetes should be much lower than that of Caucasian if the C allele plays a promoting role in the pathogenesis of type 2 diabetes [7]. However, neither the observation of the putative protective or promoting role of *IL-6* -174 C allele is likely to be applied in the pathogenesis of Taiwanese T2DM because we did not identified any *IL-6* -174 C allele in Taiwanese population.

Three possible factors could contribute to the lack of association of the *IL-6* genotype with type 2 diabetes in the Taiwanese population: a non-causative effect of the G allele as mentioned above, linkage disequilibrium of the G allele with a nearby non-causative polymorphism, or confounding due to either ethnic admixture or other source(s) of population stratification because population stratification may underlie the findings. The association between T2DM and G alleles can arise when the disease and the *IL-6* G allele are both associated with ethnic origin. For example, the G allele was associated with Native American individuals with full Pima heritage; therefore, only the G allele was observed among full heritage Pima Indians who have very high prevalence of type 2 diabetes [11]. Similarly, a higher frequency of the GG genotype was observed in Afro-Caribbean and Gujarati Indians, both of whom also have high type 2 diabetes prevalence [8, 12]. In those cases, the disease and allele will be associated in persons whose heritage is a mixture of these groups even when the allele is not causal. It is apparently unlikely that the *IL-6* -174 polymorphism is the only susceptible or protective factor of type 2 diabetic development, in addition, we can not rule out the possibility that linkage disequilibrium of the G alleles, with a nearby non-causative polymorphism leads to our observations at the present stage because all of our diabetic and control subjects were of full Taiwanese heritage. Nevertheless, the absence of the C allele in our healthy and type 2 diabetic individuals suggested that this polymorphism did not affect the development of this disease in Taiwanese people while the WBC count, a subclinical inflammatory marker, was significantly higher among our patients than among the control individuals ($P < 0.001$, *t*-test). In summary, our results reflected the unique genetic characteristics and possible distinct etiological/environmental factors that may be involved in the pathogenesis of Taiwanese T2DM.

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