Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 1717–1722
Metabolism
Clinical and Experimental
www.metabolismjournal.com

# Association of interleukin-4 promoter polymorphisms in Taiwanese patients with type 2 diabetes mellitus

Kuo-Ting Ho[a,1], Ming-Yuh Shiau[b,1], Yih-Hsin Chang[c,d,1], Chuan-Mu Chen[a,1], Shun-Chun Yang[c], Chien-Ning Huang[e,f,*]

[a]*Department of Life Sciences, National Chung Hsing University, Taichung 402, Taiwan, ROC*
[b]*Hungkuang University, Taichung 433, Taiwan, ROC*
[c]*School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung 402, Taiwan, ROC*
[d]*Clinical Laboratory, Chung Shan Medical University Hospital, Taichung 402, Taiwan, ROC*
[e]*Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung 402, Taiwan, ROC*
[f]*School of Medicine, Chung Shan Medical University, Taichung 402, Taiwan, ROC*

Received 10 November 2009; accepted 12 April 2010

**Abstract**

Many factors have been implicated in the onset of type 2 diabetes mellitus (T2DM). Recently, immune response and inflammation were suggested to play certain roles in the development and complications of T2DM. The aim of this study is to investigate the putative correlation between the promoter polymorphisms of interleukin-4 (IL-4), one of the immune-regulatory type 2 helper T-cell cytokines, and T2DM. Genomic DNA from 425 Taiwanese T2DM patients and 148 nondiabetic control study subjects were extracted, and their IL-4 promoter polymorphisms were analyzed by polymerase chain reaction–restriction fragment length polymorphism. Both of the distribution of IL-4 C-589T ($P$ = .013) and C-34T ($P$ = .05) genotypes were significantly different between T2DM patients and control subjects. Significant association between IL-4 C-589T alleles ($P$ = .002) and T2DM, as well as C-34T alleles and T2DM ($P$ =.024), was also identified. In addition, a statistically significant association between homologous IL-4 −589 C/C genotype and lower circulatory high-density lipoprotein cholesterol levels was observed. Our results suggested that IL-4 promoter polymorphisms are associated with T2DM. A significant association between IL-4 −589 C/C genotype and lower circulatory high-density lipoprotein cholesterol level was observed as well. The above results suggested that IL-4 may participate in lipid metabolism and diabetic susceptibility.


## 1. Introduction

Type 2 diabetes mellitus (T2DM) is caused by insulin resistance and often combined with symptoms of progressive defect in insulin secretion. The number of the diabetic patients is rapidly increasing globally, with an estimated increased rate of 46% from year 2000 to 2010. The prevalence of T2DM varies among different ethic populations, with the highest rate found in Pima Indians (as high as ~50 [1]). Type 2 diabetes mellitus prevalence in the Taiwanese population (about 1.5%) is much lower than that in whites (4~16%) [2]. The discrepancy indicates that unique genetic characteristics and possibly distinct etiologic/environmental factors may be involved in the pathogenesis of T2DM in Taiwan.

Many factors can lead to T2DM onset; however, host genetic factors are the focus of discussion. Crook et al [3] and Pickup et al [4] first proved that T2DM is an inflammatory condition characterized by elevated concentrations of acute phase inflammatory reactants in the plasma. These acute phase proteins are synthesized in liver and stimulated by cytokines, mainly interleukin-1 (IL-1), IL-6, and tissue necrosis factor-α. The circulatory IL-6 levels in T2DM patients are also increased, despite the basal production of IL-6 in cultured diabetic blood cells being markedly depressed [4,5]. Consequently, T2DM is

* Corresponding author. Department of Internal Medicine, Chung Shan Medical University Hospital, School of Medicine, Chung Shan Medical University, Taichung 402, Taiwan. Tel.: +886 4 2473 9595x34701; fax: +886 4 2473 9220.
*E-mail address:* cshy049@csh.org.tw (C.-N. Huang).
[1] Equal contribution to this study.

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.04.010

an acute phase disease in which increased concentrations of cytokines contributing to the regulation of balance between Th1 and Th2 cells are involved [6,7]. These studies implicate that acute inflammation will result in glucose intolerance and diabetes, and many of the clinical and biochemical T2DM features as well as its complications may be explained by the augmented acute phase response. Moreover, many observations suggest that diabetes may be associated with enhanced cytokine production, raising the possibility that some of the diabetes-associated metabolic abnormalities may be due to or exacerbated by cytokine overproduction [8].

Accordingly, immune response and inflammation are suggested to play certain roles in the development and complications of T2DM. Type 2 T helper cell (Th2) cytokine IL-6, which contributes to the exquisite regulation of Th1/Th2 balance, is one of the well-studied cytokines in diabetic research (reviewed by Paul and Seder [7]). In addition to IL-6, other cytokines that affect the Th1/Th2 balance might also participate in T2DM development. Therefore, it is intriguing to investigate if other Th2 cytokines are involved in the pathogenesis of T2DM. Nevertheless, studies regarding the correlation between Th2 cytokines other than IL-6 and T2DM are limited.

Interleukin-4, mainly secreted by activated T cells, $Fc\varepsilon R1^{+}$ cells, and eosinophils, is an important anti-inflammatory cytokine that can inhibit the secretion of the proinflammatory cytokines from macrophages [9,10]. The production of IL-4 is tightly controlled at the level of gene transcription [11]. Several single nucleotide polymorphisms (SNPs) have been identified in the promoter region of the IL-4 gene, such as the SNPs located at positions −589 (C to T), −285 (C to T), −81 (A to G), and −34 (C to T) from the transcription start site [12-14]. These SNPs have been identified to influence promoter strength and thus mediate transcription and expression of IL-4 gene. Several studies have examined the possible correlation of IL-4 genotypes and type 1 diabetes mellitus (T1DM) pathogenesis, with the association of IL-4 SNPs and T1DM reported in some [15,16] but not all studies [17,18]. This may be, in part, the result of different ethnic populations being studied.

The putative association between IL-4 and the more prevalent T2DM did not cause much attention until a recent study demonstrated that the genetic polymorphisms of IL-4 intron-3 could serve as susceptibility indicators for T2DM in the Indian population [19]. Therefore, it is tempting to identify whether the IL-4 promoter polymorphisms that influence the transcription activity and cytokine secretion ability would contribute to T2DM pathogenesis. The present study aimed at investigating if the IL-4 gene promoter SNPs are associated with Taiwanese T2DM. To inspect this hypothesis, the inheritance of the IL-4 promoter SNPs among patients with T2DM and the association of these polymorphisms with patients' biochemical features were examined.

## 2. Materials and methods

### 2.1. Study subjects

Fasting venous blood samples were taken from 425 T2DM patients attending the diabetic clinic in the Department of Internal Medicine, Chung Shan Medical University Hospital. Fasting blood samples form 148 nondiabetic control subjects were collected from the Physical Check Up Unit, Taichung Veterans General Hospital. Written consents were obtained from all the study subjects after the nature of the procedure was explained. The information on body height, weight, age, fasting blood glucose, and renal function index (creatinine [CRE], blood urea nitrogen [BUN]), etc; listed in Table 1) were collected for further statistical analysis.

### 2.2. Analysis of IL-4 promoter polymorphisms

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs). An aliquot of the genomic DNA (50-100 ng) was used for amplifying the promoter region containing each polymorphism by polymerase chain

Table 1
Demographic and biochemical data of study subjects in this study

| | Control subjects n = 148 (n = 98)[b] | T2DM patients n = 425 (n = 328)[b] | $P$[c] |
|---|---|---|---|
| Male/female | 75/73 | 198/187 | NS |
| | (48/50) | (166/162) | NS |
| Age (y) | 57.93±10.62 | 57.24±11.44 | NS |
| | (58.16±10.91) | (57.07±11.56) | NS |
| BMI (kg/m$^2$) | 24.52±3.40 | 25.36±3.27 | .014 |
| | (24.43±3.51) | (25.34±3.16) | .021 |
| Fasting glucose | 95.69±6.79 | 177.70±68.50 | <.001 |
| (70-110 mg/dL)[a] | (96.07±6.63) | (180.38±70.65) | <.001 |
| Systolic pressure | 125.42±19.18 | 134.76±18.56 | <.001 |
| (120-140 mm Hg)[a] | (123.45±17.19) | (135.22±18.19) | <.001 |
| Diastolic pressure | 79.20±10.38 | 80.30±10.80 | NS |
| (70-90 mm Hg)[a] | (78.28±9.33) | (79.99±10.51) | NS |
| BUN | 15.86±5.09 | 17.54±8.00 | .025 |
| | (15.60±4.39) | (17.58±8.07) | .023 |
| CRE (0.6-1.4 mg/dL)[a] | 1.11±0.42 | 1.04±0.44 | NS |
| | (1.09±0.24) | (1.04±0.44) | NS |
| Cholesterol | 201.18±37.51 | 198.2±42.22 | NS |
| (125-240 mg/dL)[a] | (202.62±39.21) | (198.02±42.12) | NS |
| HDL-C (>35 mg/dL)[a] | 58.35±13.88 | 46.49±13.38 | <.001 |
| | (59.24±14.58) | (46.52±13.43) | <.001 |
| TC/HDL-C | 3.59±0.90 | 4.50±1.31 | <.001 |
| | (3.56±0.89) | (4.51±1.31) | <.001 |
| Triglycerides | 142.54±125.52 | 184.00±153.24 | .004 |
| (20-200 mg/dL)[a] | (139.45±122.86) | (184.51±153.74) | .008 |
| Uric acid | 6.56±1.64 | 6.11±1.87 | .021 |
| (2.4-7.2 mg/dL)[a] | (6.79±1.67) | (6.12±1.88) | .003 |

NS indicates nonsignificant; BMI, body mass index.

[a] Numbers in parentheses indicate the reference range of each biochemical test.

[b] Numbers in parentheses indicate the information of each demographic and biochemical variables from the data of 98 control and 328 diabetic subjects with available IL-4 −34 genotypic results.

[c] Student $t$ test.

reaction (PCR). Generally, DNA amplification was performed in a 20-$\mu$L volume containing 10 pmol of each primer, 4.5 mmol/L $MgCl_2$, 0.25 mmol/L of each dNTP, 1 unit Taq polymerase, and 1.5-mmol/L buffer with a 95°C initial incubation for 5 minutes, followed by 30 amplification cycles and a final extension. Promoter polymorphisms were examined by restriction fragment length polymorphism (RFLP). Specific primers and PCR conditions, and respective enzyme digestion and conditions in RFLP were as described previously [20] and listed in Table 2.

### 2.3. Analysis of IL-4 secretion

Peripheral blood mononuclear cells were isolated from whole blood using Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation. A total of $2 \times 10^6$ PBMCs were cultured in RPMI medium (Hyclone, South Logan, UT) containing 10% fetal bovine serum (GIBCO, Mexico). After 24 hours of 10-$\mu$g/mL concanavalin A (Con A, Sigma, Steinheim, Germany) treatment, secreted IL-4 by the activated PBMC was determined using IL-4 enzyme-linked immunosorbent assay kit (R&D, Minneapolis, MN).

### 2.4. Statistical analysis

Data analysis started with descriptive statistics, including mean and standard deviation for continuous variables and frequency for categorical variables. If necessary, natural logarithm transformation was used to enhance normality for blood biochemistry parameters with skewed distribution. Student *t* test was applied for comparisons of age, body mass index, and each of the blood biochemistry parameters between diabetic subjects and controls; and $\chi^2$ test was used for comparing frequencies of different genotypes and sex between groups. Moreover, 1-way analysis of variance was applied to compare means of respective blood biochemistry parameters among subjects with different IL-4 genotypes. Finally, multiple linear regression analysis was used to assess the associations between IL-4 genotypes and the biochemical parameters, with adjustment for diabetes status, age, and sex. The statistical software of SAS version 8 (SAS Institute, Cary, NC) was applied for the analyses. An $\alpha$ level of 0.05 was used for all statistical tests.

Table 2
The primer sequences used in the analysis of IL-4 promoter polymorphisms and reverse transcriptase PCR

| Primer designation | Primer sequence |
|---|---|
| IL4 −589F | 5′-TGGGTAAGGACCTTATGGACC -3′ |
| IL4 −589R | 5′- GGTGGCATCTTGGAAACTGTC -3′ |
| IL4 −285F | 5′-TGGGTAAGGACCTTATGGACC-3′ |
| IL4 −285R[b] | 5′-GAAGCAGTTGGGA*C*GTGAGA-3′ |
| IL4 −81F | 5′-CCAGCAGCAGCCCCAAGCTGA-3′ |
| IL4 −81R | 5′-TGCAGTGAGAATGTGAGGCAA-3′ |
| IL4 −34F[a] | 5′-CTCATTTTCC*G*TCGGTTTCAGC-3′ |
| IL4 −34R[b] | 5′-GAAGCAGTTGGGA*C*GTGAGA-3′ |

[a] One base-exchange substitution from C to G position at position −50 destroyed the *Mnl*I restriction site.

[b] One base-exchange substitution from G to C position at position +9 destroyed the *Mnl*I restriction site.

## 3. Results

Our study aimed at investigating the distribution of the IL-4 promoter SNPs among control and T2DM subjects to test the possible correlation between IL-4 genetic polymorphisms and T2DM. Polymorphisms of IL-4 C-589T were successfully investigated in all study subjects, whereas the subject numbers of other IL-4 SNPs investigated were variable, probably because of the insufficient quantity and/or quality of genomic DNA from certain study subjects. Demographic data and clinical biochemical manifestations of the patients with available genotypic data are listed in Table 1.

### 3.1. Significant association of IL-4 promoter polymorphisms with T2DM

Results regarding the distribution of the IL-4 promoter SNPs among the control and diabetic subjects are summarized in Table 3. Significant difference in distribution of IL-4 C-589T genotypes between T2DM patients and control subjects was observed ($P = .013$), as well as between IL-4 −589 alleles and T2DM ($P = .002$). In addition, significant difference of IL-4 C-34T genotypic distribution between T2DM and control individuals was also observed ($P = .05$). Although this genotypic difference only reached marginal significance, a strong association of IL-4 −34 alleles and T2DM was discovered ($P = .024$). Interestingly, no polymorphisms at IL-4 −81 and −285 positions were found. The above observations demonstrated that the IL-4 polymorphisms were associated with T2DM.

Table 3
Comparison of IL-4 −34 C/T and −589 C/T polymorphisms between T2DM subjects and controls

| Genotype/allele | Control subjects n (%) | T2DM patients n (%) | $P$[a] |
|---|---|---|---|
| −589 C/T | 148 | 425 | |
| T/T | 96 (64.86%) | 324 (76.24%) | .013 |
| T/C | 45 (30.41%) | 93 (21.88%) | |
| C/C | 7 (4.73%) | 8 (1.88%) | |
| T allele | 237 (80.07%) | 741 (87.18%) | .002 |
| C allele | 59 (19.93%) | 109 (12.82%) | |
| −34 C/T | 98 | 328 | |
| T/T | 63 (64.29%) | 250 (76.22%) | .05 |
| T/C | 32 (32.65%) | 69 (21.04%) | |
| C/C | 3 (3.06%) | 9 (2.74%) | |
| T allele | 158 (80.61%) | 569 (86.74%) | .024 |
| C allele | 38 (19.39%) | 87 (13.26%) | |
| −285 C/T | 132 | 140 | – |
| C/C | 132 (100%) | 140 (100%) | |
| −81 A/G | 125 | 114 | – |
| A/A | 125 (100%) | 114 (100%) | |

[a] $\chi^2$ test.

### 3.2. Significant association between high IL-4 secreting ability and T2DM

To further investigate the association of IL-4 and T2DM, IL-4 secreting levels of Con A–activated PBMC from study subjects were determined (Table 4). The results showed that, although the basal IL-4 levels were similar in control and T2DM groups (0~8 pg/mL, data not shown), IL-4 secreting level after Con A stimulation was significantly higher in T2DM patients (19.01 ± 1.27 pg/mL, n = 106) compared with that in control subjects (5.44 ± 1.42 pg/mL, n = 26, $P <$ .001). The results indicated that PBMC from T2DM patients had higher IL-4 secreting ability.

### 3.3. Association of homologous IL-4 −589 C/C genotype with high-density lipoprotein cholesterol levels and the ratio of total cholesterol to high-density lipoprotein cholesterol

In addition to the significant association between IL-4 C-589T polymorphisms with T2DM, we further investigated the correlation between this polymorphism and study subjects' biochemical data using multiple linear regression analysis adjusted for age, sex, and diabetes status (Table 5). When the biochemical data of individuals carrying C/C genotype were compared with those of individuals carrying T/T genotype, a significant difference was found in high-density lipoprotein cholesterol (HDL-C) ($P = <.01$) and the ratio of total cholesterol (TC) to HDL-C ($P < .01$). These findings indicated that individuals with IL-4 −589 C/C genotype tend to have lower HDL-C (parameter estimate [standard error; SE] = −0.42) and higher TC/HDL-C (parameter estimate [SE] = 0.41). No significant difference of the biochemical data between subjects with T/C genotype and T/T genotype was found. The results implied that IL-4 −589 C/C genotype might be associated with HDL-C metabolism and, therefore, contribute to the observed difference of TC/HDL-C.

## 4. Discussion

Cytokines, secreted by a variety of activated cells and acting as regulators of immune responses, are believed to be involved in immune response–induced destruction of islet β-cells in T1DM [21]. In animal models, transgenic expression of IL-4 in β-cells [22] and systemic IL-4 administration [23] can prevent NOD mice from insulitis and diabetes. Interleukin-4 is suggested to protect human islets from cytotoxic damage induced by proinflammatory and Th1 cytokines [24]. However, IL-4 expression is reported not to correlate with destructive and benign insulitis in T1DM patients [25]. Another study shows that long-term exposure of rat pancreatic islets to IL-4 results in an inhibitory action to some of the islet functions [26], probably due to an influence on the islet glucose metabolism.

Several studies have documented the correlations between the genotypes of IL-4/IL-4 receptor (IL-4R) and diabetic status. Mirel et al (n = 282) [27] and Bugawan et al (n = 90) [16] revealed that IL-4R genotypes were associated with protection from T1DM; however, no significance was observed between IL-4 SNP and T1DM. On the contrary, other studies suggested that IL-4R genotype were not associated with T1DM [18,28-30]. Possible factors that contribute to the above conflicting results include linkage disequilibrium of IL-4 and/or IL-4R gene to a nearby noncausative polymorphism, or confounding due to either ethnic admixture or the source(s) of population stratification. Besides, sample size is also suggested to be one of the major factors that affect the results and interpretation.

Despite the inconsistent conclusions concerning the association between IL-4 and T1DM, it is intriguing to examine if IL-4 is involved in the more prevalent T2DM that has been proven to be closely associated with inflammation. However, very little is known regarding this issue. The major finding in the present study was the significant difference in the IL-4 −589 genotypic distribution between healthy and T2DM individuals ($P$ = .002, Table 3). It suggested that individuals with IL-4 high-secreting genotypes might be more predisposed to T2DM. Individuals carrying either heterozygous or homozygous IL-4 −589 T allele are reported to be associated with higher IL-4 secreting ability [31,32]. Gervaziev et al [33] demonstrated that higher serum IL-4 levels are associated with IL-4 −34 T and −589 T alleles in the Russian population. It is also reported [34] that coronary artery bypass grafting patients with IL-4 −589 T/T genotype had significantly higher circulating levels of IL-4 postoperatively compared with patients with C/C and C/T genotypes. The observation that T2DM patients have higher IL-4 secreting ability (Table 4) further supported our speculation.

Table 4
Interleukin-4 secreting levels of activated PBMC from control and T2DM subjects

| | Control subjects n = 26 | T2DM n = 106 | $P$[a] |
|---|---|---|---|
| Mean concentrations (pg/mL) | 5.44 ± 1.42 | 19.01 ± 1.27 | <.001 |

[a] Wilcoxon test.

Table 5
Association of IL-4 −589 genotypes and blood chemistry parameters

| Outcome | IL-4 genotypes (C/C vs T/T) | | IL-4 genotypes (T/C vs T/T) | |
|---|---|---|---|---|
| | $B$ (SE)[a] | $P$ value | $B$ (SE)[a] | $P$ value |
| Fasting glucose | 0.04 | .68 | 0.02 | .55 |
| BUN | 0.22 | .15 | 0.05 | .39 |
| CRE | 0.09 | .30 | <0.01 | .93 |
| Cholesterol | <0.01 | .10 | 0.02 | .49 |
| HDL-C | −0.42 | <.01 | −0.02 | .67 |
| TC/HDL-C | 0.41 | <.01 | 0.08 | .13 |
| Triglyceride | −0.34 | .24 | 0.14 | .16 |
| Uric acid | 0.01 | .96 | 0.01 | .80 |

[a] Parameter estimate (standard error).

In addition, we suggested that the homologous IL-4 −589 C/C genotype was associated with HDL-C metabolism, according to the linear regression results adjusted by variables of age, sex, and diabetic status (Table 5). It is intriguing to explain the observation that subjects carrying lower IL-4 secreting −589 C/C genotype would have lower peripheral HDL-C levels, which is independent of diabetic status. As a matter of fact, IL-4 is known to be correlated with atherosclerosis [35-37]. In a mice model, severe hypercholesterolemia is associated with a switch to Th2 immune response, with increased IL-4 expression in the lesions [35]. Interleukin-4 messenger RNA can also be detected in atherosclerotic lesions in human body [36]. The microenvironmental IL-4 in the atherosclerotic lesions has multiple effects on atherogenesis, such as the augmentation of low-density lipoprotein cholesterol esterification by a concentration- and time- dependent manner [37]. In addition, IL-4 can regulate the expression of 15-lipoxygenase, a key enzyme in low-density lipoprotein oxidation [38,39]. Elbe-Bürger et al [40] further demonstrated that the adipocyte layer in the dermis is reduced in IL-4 transgenic mice. Moreover, by exploring the influence of IL-4 to fatty streak formation, George et al [41] found that HDL and triglycerides in IL-4–deficient mice were higher. The above studies indicate that local microenvironmental expression of IL-4 is involved in lipid metabolism and eventually the atherogenic process. Accordingly, we speculated that, although individuals with IL-4 −589 T/T or C/T genotypes were more susceptible to diabetic onset, diabetic individuals with IL-4 −589 C/C genotype might be more susceptible to diabetic complications such as atherosclerosis and cardiovascular diseases. However, whether the interindividual differences in the IL-4 secretion levels contribute to the HDL-C metabolism and predisposition of diabetic complications needs further investigation.

The IL-4 promoter SNPs at positions −285 (C/T), −81 (A/G), and −34 among our patients were also characterized. Interestingly, no polymorphisms at positions −285 and −81 were found in our population. The IL-4 −34 C/T polymorphisms are reported to be associated with −589 C/T SNPs [13]; that is, −34 C allele and T allele are always associated with −589 C allele and T allele, respectively. Therefore, the observed significance between IL-4 −34 SNPs and T2DM might be in part a result of the linkage disequilibrium between −34 and −589 SNPs.

In addition to SNPs, IL-4 gene expression is tightly regulated at the level of transcription by multiple transcription factors that bind to *cis*-elements in the upstream proximal promoter. The promoter activity of A to G transition at −81 will enhance 8-fold because this transition makes the promoter a higher affinity binding to AP-1–specific transcription factors [14]. To the best of our knowledge, no transcription factors are reported to bind to IL-4 −589 promoter region. Therefore, it is also intriguing to investigate if there is any transcription factor(s) that can bind to this region. Once the candidate transcription factors are found, the binding capacity of the proteins to different genotypes and the resultant transcription ability of IL-4 promoter, as well as the effect of this SNP to HDL-C metabolism, can subsequently be determined.

In summary, we have demonstrated that IL-4 promoter polymorphisms are significantly associated with T2DM. Moreover, an association between homologous IL-4 −589 C/C genotype and lower HDL-C level is characterized in our study. Our results suggest that polymorphisms of the IL-4 promoter may contribute to the HDL-C metabolism, T2DM predisposition, and diabetic complications in Taiwanese subjects.

## Acknowledgment

This work was supported by National Science Council, Taiwan, Republic of China (NSC 97-2320-B-040-006-MY3).

## References

[1] Chang YH, Huang CN, Shiau MY. The C-174G promoter polymorphism of the IL-6 gene that affects insulin sensitivity in Caucasians is not involved in the pathogenesis of Taiwanese type 2 diabetes mellitus. Eur Cytokine Netw 2004;15:117-9.

[2] Shiau MY, Huang CN, Wu CY, et al. Association of IL-10 A-592C and T-819C polymorphisms with type 2 diabetes mellitus. Hum Immunol 2005;66:1258-63.

[3] Crook MA, Tutt P, Pickup JC. Elevated serum sialic acid concentration in NIDDM and its relationship to blood pressure and retinopathy. Diabetes Care 1993;16:57-60.

[4] Pickup JC, Mattock MB, Chuaney GD, et al. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 1997;40:1286-92.

[5] Tsiavou A, Hatziagelaki E, Chaidaroglou A, et al. TNF-alpha, TGF-beta1, IL-10, IL-6, gene polymorphisms in latent autoimmune diabetes of adults (LADA) and type 2 diabetes mellitus. J Clin Immunol 2004;24:591-9.

[6] Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 1998;41:1241-8.

[7] Paul WE, Seder RA. Lymphocyte responses and cytokines. Cell 1994;76:241-51.

[8] Feingold KR, Grunfeld C. Role of cytokines in inducing hyperlipidemia. Diabetes 1992;41(Suppl 2):97-101.

[9] Paul WE. Interleukin 4: signaling mechanisms and control of T cell differentiation. Ciba Found Symp 1997;204:208-16.

[10] Garcia-Zepeda EA, Combadiere C, Rothenberg ME, et al. Human monocyte chemoattractant protein-4 is a novel CC chemokine with activates on macrophage, eosinophils, and basophils induced in allergic and non-allergic inflammation that signals through the CC chemokine receptors (CCR)–2 and –3. J Immunol 1996;157:5613-26.

[11] Georas S, Cumberland J, Burke T, et al. Characterization of a novel negative regulatory element in the human interleukin 4 promoter. Leukemia 2000;14:629-35.

[12] Rosenwasser LJ, Klemm DJ, Dresback JK, et al. Promoter polymorphism in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allergy 1995;25(Suppl 2):74-8.

[13] Song Z, Casolaro V, Chen R, et al. Polymorphic nucleotides within the human IL-4 promoter that mediate overexpression of the gene. J Immunol 1996;156:424-9.

[14] Takabayashi A, Ihara K, Sasaki Y, et al. Novel polymorphism in the 5′-untranslated region of the interleukin-4 gene. J Hum Genet 1999;44:352-3.

[15] Steck AK, Bugawan TL, Valdes AM, et al. Association of non-HLA genes with type 1 diabetes autoimmunity. Diabetes 2005;54:2482-6.
[16] Bugawan TL, Mirel DB, Valdes AM, et al. Association and interaction of the IL-4R, IL-4, and IL13 loci with type 1 diabetes among Filipinos. Am J Hum Genet 2003;72:1505-14.
[17] Nuñez C, Santiago JL, Varadé J, et al. IL-4 in the 5q31 context: association studies of type 1 diabetes and rheumatoid arthritis in the Spanish population. Immunogenetics 2008;60:19-23.
[18] Reimsnider SK, Eckenrode SE, Marron MP, et al. IL4 and IL4Ralpha genes are not linked or associated with type 1 diabetes. Pediatr Res 2000;47:246-9.
[19] Bid HK, Konwar R, Agrawal CG, et al. Association of IL-4 and IL-1RN (receptor antagonist) gene variants and the risk of type 2 diabetes mellitus: a study in the north Indian population. Indian J Med Sci 2008; 62:259-66.
[20] Shiau MY, Huang CN, Yang TP, et al. Cytokine promoter polymorphisms in Taiwanese patients with Graves' disease. Clin Biochem 2007;40:213-7.
[21] Rabinovitch A, Suarez-Pinzon WL. Cytokines and their roles in pancreatic islet $\beta$-cell destruction and insulin-dependent diabetes mellitus. Biochem Pharmacol 1998;55:1139-49.
[22] Mueller R, Krahl T, Sarvetnick N. Pancreatic expression of interleukin-4 abrogates insulitis and autoimmune diabetes in nonobese diabetic (NOD) mice. J Exp Med 1996;184:1093-9.
[23] Rapoport MJ, Jaramillo A, Zipris D, et al. Interleukin 4 reverses T-cell proliferative unresponsiveness and prevents the onset of diabetes in NOD mice. J Exp Med 1993;178:87-99.
[24] Marselli L, Dotta F, Piro S, et al. Th2 cytokines have a partial, direct protective effect on the function and survival of isolated human islets exposed to combined proinflammatory and Th1 cytokines. J Clin Endocrinol Metab 2001;86:4974-8.
[25] Rabinovitch A. An update on cytokines in the pathogenesis of insulin dependent diabetes mellitus. Diabetes Metab Rev 1998;14:129-51.
[26] Sandler S, Sternesjö J. Interleukin 4 impairs rat pancreatic islet function in vitro by an action different to that of interleukin 1. Cytokine 1995;7:296-300.
[27] Mirel DB, Valdes AM, Lazzeroni LC, et al. Association of IL4R haplotypes with type 1 diabetes. Diabetes 2002;51:3336-41.
[28] Maier LM, Twells RC, Howson JM, et al. Testing the possible negative association of type 1 diabetes and atopic disease by analysis of the interleukin 4 receptor gene. Genes Immun 2003;4:469-75.
[29] Maier LM, Chapman J, Howson JM, et al. No evidence of association or interaction between the IL4RA, IL4, and IL13 genes in type 1 diabetes. Am J Hum Genet 2005;76:517-21.
[30] Erlich HA, Lohman K, Mack SJ, et al. Type I Diabetes Genetics Consortium. Association analysis of SNPs in the IL4R locus with type I diabetes. Genes Immun 2009;10(Suppl 1):S33-S41.
[31] Noguchi E, Shibasaki M, Arinami T, et al. Association of asthma and the interleukin-4 promoter gene in Japanese. Clin Exp Allergy 1998; 28:449-53.
[32] Noguchi E, Nukaga-Nishio Y, Jian Z, et al. Haplotypes of the 5′ region of the IL-4 gene and SNPs in the intergene sequence between the IL-4 and IL-13 genes are associated with atopic asthma. Hum Immunol 2001;62:1251-7.
[33] Gervaziev YV, Kaznacheev VA, Gervazieva VB. Allelic polymorphisms in the interleukin-4 promoter regions and their association with bronchial asthma among the Russian population. Int Arch Allergy Immunol 2006;141:257-64.
[34] Bittar MN, Khasati NH, Deiraniya AK, et al. Interleukin-4 C-590T polymorphism has no role in coronary artery bypass surgery. Asian Cardiovasc Thorac Ann 2007;15:214-7.
[35] Zhou X, Paulsson G, Stemme S, et al. Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. J Clin Invest 1998; 101:1717-25.
[36] Stemme S, Faber B, Holm J, et al. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci USA 1995;92:3893-7.
[37] Cornicelli JA, Butteiger D, Rateri DL, et al. Interleukin-4 augments acetylated LDL-induced cholesterol esterification in macrophages. J Lipid Res 2000;41:376-83.
[38] Conrad DJ, Kuhn H, Mulkins M. Specific inflammatory cytokines regulate the expression of human monocyte 15-LO. Proc Natl Acad Sci USA 1992;89:217-21.
[39] Cornicelli JA, Welch K, Auerbach B. Mouse peritoneal macrophages contain abundant v-6 lipoxygenase activity that is independent of interleukin-4. Arterioscler Thromb Vasc Biol 1996;16:1488-94.
[40] Elbe-Bürger A, Egyed A, Olt S, et al. Overexpression of IL-4 alters the homeostasis in the skin. J Invest Dermatol 2002;118:767-78.
[41] George J, Mulkins M, Shais A, et al. Interleukin (IL)-4 deficiency does not influence fatty streak formation in C57BL:6 mice. Atherosclerosis 2000;153:403-11.