Research Article

Association of *IL*-4-590 *C*>*T* and *IL*-13-1112 *C*>*T* Gene Polymorphisms with the Susceptibility to Type 2 Diabetes Mellitus

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Background. The goal of the study is to investigate the association of *IL*-4-590 and *IL*-13-1112 genetic polymorphisms with type 2 diabetes mellitus (T2DM) in Egyptian patients. *Subjects and Methods.* The study included 135 cases with T2DM and 75 healthy unrelated age-matched controls from the same locality of Egypt. DNA was extracted and processed by the ARMS-PCR technique for characterization of genetic variants of *IL*-4-590 *C*>*T* and *IL*-13-1112 *C*>*T* polymorphisms. *Results.* Egyptian cases with T2DM showed a lower frequency of the *IL*-4-590 *CC* homozygous genotype compared to controls (10.4% versus 43.48%) with a higher *CT* heterozygous genotype (85.2% versus 47.8%). Similarly, cases showed a lower frequency of the *IL*-13-1112 *CC* genotype (20.7% versus 56.8%) with a higher frequency of the heterozygous *IL*-13-1112 *CT* genotype (76.3% versus 41.3%). Both polymorphisms showed significantly positive associations with T2DM in the dominant, codominant, and overdominant models of inheritance. On the other hand, comparing genotypes of subgroups related to gender, positive family history, and positive consanguinity showed a nonsignificant difference ($P > 0.05$). *Conclusion.* Heterozygous genotypes (*IL*-4-590 *CT* and *IL*-13-1112 *CT*) could be considered as risk factors, while the homozygous wild types (-590 *CC* and -1112 *CC*) might be considered protective to T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is believed to be a multifactorial disease that is influenced by genetic and environmental factors. People with a family history of the T2DM are at a higher risk of developing the disease since they share genetic background in addition to likely similar environments [1].

Interleukin 4 (*IL-4*) was originally discovered as a low molecular weight T cell-derived polypeptide of 129 amino acids, which is encoded by the *IL-4* gene on chromosome 5q23.31. It is secreted by helper T cells (CD4) type 2 (Th2) lymphocytes, and natural killer (NK) T cells, and by cells of the innate immune system, including mast cells, basophils, and eosinophils [2]. Interleukin 4 regulates proliferation, apoptosis, gene expression, and differentiation in many hematopoietic cells; in particular, it directs the Ig class switch to IgG1 and IgE and downregulates the production of Th1 cells [3, 4]. *IL-4* is suggested to protect human islets from cytotoxic damage induced by proinflammatory and Th1 cytokines. Another study showed that a long-term exposure of rat pancreatic islets to *IL-4* resulted in an inhibitory action to some of the islet functions [5].

Interleukin 13 is a 12-kDa-protein product, produced by Th2 cells which are genetically implicated in the pathogenesis of inflammatory and immune systemic diseases, such as asthma and atopy. IL-13 shares several biological profiles with *IL-4* including IgE production, CD23 and MHC class II expression, and inhibition of antibody-dependent cell-mediated cytotoxicity with downregulation of IgG type I receptor and suppression of type I interferon [6].

Genetic ablation of IL-13 in mice resulted in hyperglycemia, which progressed to hepatic insulin resistance and systemic metabolic dysfunction [7]. The previous functional studies of IL-13 and its SNPs strongly suggest that IL-13 is the causal gene for raised IgE levels and atopy risk. Moreover, IL-13 alleles that are predisposing to high IgE levels (part of Th2 responses) might show an inverse effect on Th1 associated type 1 diabetes [8].

The aim of the present work is to investigate the association of polymorphisms of *IL-4-590 C>T* and *IL-13-1112 C>T* genes with the susceptibility and clinical pattern of T2DM among Egyptian cases from the Nile Delta region of Egypt.

2. Subjects and Methods

This is a case-controlled study that was conducted on 135 type 2 diabetic patients and 75 healthy controls recruited during the time period from May 2012 to July 2013. Patients were recruited from the Outpatient Clinics and Inpatient Department of Diabetes and Endocrinology Unit, Specialized Medical Hospital, Mansoura University, Egypt. They included 65 males and 70 females with a mean age of 56.11 years (SD = 9.1). All patients had a diagnosis of established T2DM on the basis of the criteria developed by the World Health Organization [9]. Seventy-five healthy unrelated subjects with mean age of 59.0 years (SD = 1.0) from the same locality were used as the controls. An informed consent was taken from all participants before the study. In addition, an approval was obtained from the ethical and scientific committees of the local health authorities.

2.1. Genotyping of IL-4-590 C>T and IL-13-1112 C>T Polymorphisms. For all participants, DNA was isolated from whole blood according to the Generation DNA purification capture column kit (Fermentas, #K0721, USA). Detection of the *IL-4-590 C>T* gene polymorphism (rs2243250) was done by the allele-specific PCR (ARMS-PCR) technique as described elsewhere [10]. In brief, for each person, two reactions were carried out with each of the forward primers: *IL-4* T primer: 5′-ACA CTA AAC TTG GGA GAA CAT TGT T-3′ or *IL-4* C primer: 5′-ACA CTA AAC TTG GGA GAA CAT TGT C-3′. Each of the two reactions contained the reverse primer *IL-4* 5′-GAA TTT GTT AGT AAT GCA GTC CTC C-3′. Reactions contained 5 μL of each primer, 10 μL of Dream Taq Green PCR Master Mix (2X) (Fermentas, K 1081, USA) to a final volume of 25 μL, and amplification was performed on thermal cycler with 1 min at 96°, followed by 10 cycles of 95° for 15 s, 65° for 50 s, 72° for 40 s, and 72° for 40 s; 20 cycles then 20 cycles of 95° for 50 s, 59° for 50 s, and 72° for 50 s. On the other hand, detection of the *IL-13-1112 C>T* gene polymorphism (rs1800925) was carried out using the ARMS-PCR method as described elsewhere [11]. In brief, for each person, two reactions were carried out with each of the forward primers: IL-13-1046F C primer: 5′-TTC TGG AGG ACT TCT AGG AAA AC-3′ or IL-13-1046F T primer: 5′-TTC TGG AGG ACT TCT AGG AAA AT-3′. Each of the two reactions contained the reverse primer IL-13-740R: 5′-GGA GAT GGG GTC TCA CTA TG-3′. Reactions contained

5 μL of each primer, 12 μL Dream Taq DNA Master Mix (2X, Fermentas, K1081, USA), to a final volume of 27 μL; amplification was performed on thermal cycler with 2 min at 94°C, 15 cycles of 30 s at 94°C, 30 s at 94°C, and 60 s at 72°C, 20 cycles of 30 s at 94°C, 60 s at 60°C, and 60 s at 72°C; then, PCR products were electrophoresed and visualized and photographed under UV transillumination.

2.2. Statistical Analysis. Data were processed and analyzed using the Statistical Package of Social Science (SPSS, version 17). The frequency of studied allelic polymorphism among cases was compared to that of controls describing number and percent of each and tested for positive association using Fisher's exact test and odds ratio (OR) with 95% confidence intervals (CI). Association was tested in different models of inheritance including the recessive model through comparing the homozygosity for the rare allele against others and the dominant model through comparing the combined homozygous and heterozygous variants of the rare allele against others. On the other hand, comparing heterozygous and homozygous variants each separately against the wild type variant determined the codominant model, whereas comparing the heterozygous variant only against others will determine the overdominant model of inheritance. In addition, Hardy Weinberg equilibrium (HWE) was assessed through comparison between observed and expected frequencies of genotypes related to studied polymorphisms. A minimum level of $P < 0.05$ is considered significant.

3. Results

Egyptian cases with T2DM showed a lower frequency of the *IL-4-590 CC* homozygous genotype compared to controls (10.4% versus 43.48%) with a higher *CT* heterozygous genotype (85.2% versus 47.8%). The high frequency of *IL-4-590* heterozygosity resulted in a significant positive association with T2DM in the dominant (OR = 6.65, $P = 0.000$), codominant (OR = 7.50, $P = 0.000$), and overdominant (OR = 6.27, $P = 0.000$) models of inheritance. Similarly, T2DM cases showed a lower frequency of the *IL-13-1112 CC* genotype (20.7% versus 56.8%) with a higher frequency of the heterozygous *IL-13-1112 CT* genotype (76.3% versus 41.3%). This high frequency of *IL-13-1112* heterozygosity resulted also into a significant positive association with T2DM in the dominant (OR = 4.86, $P = 0.000$), codominant (OR = 4.98, $P = 0.000$), and overdominant (OR = 4.57, $P = 0.000$) models of inheritance (Table 1). Hardy Weinberg equilibrium (HWE) showed a nonsignificant difference ($P > 0.05$) between the observed and expected frequencies of genotypes of the controls that might support the selection of controls. In contrast, HWE showed a significant deviation of the observed frequencies of cases from the expected ones, probably due to the high frequency of heterozygotes on the expense of homozygote genotypes (Table 1).

It is interesting to note that 78 cases (57.7%) had a positive family history of T2DM, while 15 cases (11.1%) had positive parental consanguinity. Comparing subgroups in terms of gender, family history, and consanguinity in relation

TABLE 1: *IL-4-590 C>T* and *IL-13-1112 C>T* genetic variants among Egyptian cases with T2DM compared to controls.

	Cases n (%)	Controls n (%)
IL-4-590 C>T		
CC	14 (10.4)	30 (43.48)
CT	115 (85.2)	33 (47.83)
TT	6 (4.4)	6 (8.69)
Dominant		
TT+CT versus *CC*	$P = 0.000^{**}$, OR = 6.65	95% CI = 3.4–13.2
Codominant		
CT versus *CC*	$P = 0.000^{**}$, OR = 7.50	95% CI = 3.7–14.9
TT versus *CC*	$P = 0.400$, OR = 2.14	95% CI = 0.6–7.7
Recessive		
TT versus *CT+CC*	$P = 0.400$, OR = 0.49	95% CI = 0.2–1.5
Overdominant		
CT versus *CC+TT*	$P = 0.000^{**}$, OR = 6.27	95% CI = 3.3–11.9
HWE	$\chi^2 = 68.0$, $P = 0.000^{**}$	$\chi^2 = 0.54$, $P = 0.462$
IL-13-1112 C>T		
CC	28 (20.7)	42 (56.00)
CT	103 (76.3)	31 (41.33)
TT	4 (3.0)	2 (2.67)
Dominant		
TT+CT versus *CC*	$P = 0.000^{**}$, OR = 4.86	95% CI = 2.7–8.8
Codominant		
CT versus *CC*	$P = 0.000^{**}$, OR = 4.98	95% CI = 2.7–9.1
TT versus *CC*	$P = 0.400$, OR = 3.0	95% CI = 0.6–16.4
Recessive		
TT versus *CT+CC*	$P = 0.760$, OR = 1.11	95% CI = 0.2–6.2
Overdominant		
CT versus *CC+TT*	$P = 0.000^{**}$, OR = 4.57	95% CI = 2.5–8.2
HWE	$\chi^2 = 44.7$, $P = 0.000^{**}$	$\chi^2 = 1.8$, $P = 0.180$

Some subjects could not be genotyped for technical reasons.
$^{**}P$ highly significant < 0.001; HVE: Hardy Weinberg Equilibrium.

to their genotype frequencies of *IL-4-590 C>T* and *IL-13-1112 C>T* polymorphic variants was found to be statistically nonsignificant ($P > 0.05$) (Table 2).

4. Discussion

Investigators of the field of immunology believe that T2DM is associated with cytokine imbalance and altered Th2 to Th1 immune response pattern [12, 13]. In addition, polymorphisms in the *IL-4 R*, *IL-4*, and the *IL-13* loci have been reported to be involved with various immune disorders as well as the regulation of serum immunoglobulin levels [6]. Therefore, we were interested to study the genetic polymorphisms of *IL-4* and *IL-13* genes that are mainly involved in the Th2 immune response pattern. In this study, we have found that T2DM in Egyptian patients was mainly positively associated with the heterozygous *CT* variants of the *IL-4-590* and *IL-13-1112* polymorphisms, that is, conforming to the codominant and overdominant models of inheritance. On the other hand, the homozygous variants *IL-4-590 CC*

and *IL-13-1112 CC* genotypes seemed to be low risk ones. This finding is in agreement with the study of Ho et al., who have reported a significant increase of the frequency of *IL-4-590 CT* heterozygous polymorphism among Taiwanese cases with T2DM [14]. Another study has also documented the association of *IL-4* and *IL-1RN VNTR* polymorphism with increased risk of T2DM in the north Indian population [15]. Their findings suggested that genetic polymorphism of both *IL-4* and *IL-RN* may finally influence initiation and progression of T2DM. Nonetheless, other studies have reported a negative association between *IL-4-590 C>T* and T2DM in Iranian subjects [16]. This might be related to ethnic genetic difference with different gene-gene interactions or due to technical errors in sampling and sorting of cases. Regarding *IL-13-1112 C>T* genetic polymorphisms, an interesting finding was the very low frequency of the *TT* homozygosity among T2DM Egyptian cases compared to controls. To our knowledge, this is the first report of a probable association between this polymorphism and T2DM that also confirmed the theory of an enhanced proinflammatory reaction towards the Th1 response [17]. The low frequency of the *IL-13-1112 C*

TABLE 2: Demographic and clinical parameters of Egyptian cases with T2DM related to genotypic variants of *IL-13-1112 C>T* and *IL-4-590 C>T* polymorphisms.

	IL-4-590 C>T			IL-13-1112 C>T		
	CC	CT	TT	CC	CT	TT
Gender						
Male	15 (23.1)	50 (76.9)	0 (.0)	8 (12.3)	53 (81.5)	4 (6.2)
Female	13 (18.6)	53 (75.7)	4 (5.7)	6 (8.6)	62 (88.6)	2 (2.9)
P	$P = 0.1320$			$P = 0.4787$		
Family history						
Positive	18 (23.1)	59 (75.6)	1 (1.3)	5 (6.4)	70 (89.7)	3 (3.8)
Negative	10 (17.5)	44 (77.2)	3 (5.3)	9 (15.8)	45 (78.9)	3 (5.3)
P	$P = 0.3233$			$P = 0.1833$		
Consanguinity						
Positive	3 (20.0)	12 (80.0)	0 (0.0)	2 (13.3)	11 (73.3)	2 (13.3)
Negative	25 (20.8)	91 (75.8)	4 (3.3)	12 (10.0)	104 (86.7)	4 (3.3)
P	$P = 0.7654$			$P = 0.1808$		

and *T* allele homozygosity in cases of T2DM is contrasting to what was found in allergic diseases as bronchial asthma that is characterized by enhanced Th2 response with high frequency of the -1112 *T* allele and a high IgE level [18, 19]. Another interesting finding was also reported by Maier et al. of a lack of an association of *IL*-4 and *IL*-13 polymorphisms with type 1 diabetes mellitus (T1DM) in white British population [8]. This finding surely points to the different immunopathology of the two diseases particularly noting that T1DM is an autoimmune disease with an underlying mechanism which is different from the immune modulation and insulin resistance impacting the presentation of T2DM [20–22]. However, taking into consideration the study limitations, particularly related to its relatively small sample size, we recommend another wider scale study with a higher number of cases and controls to investigate all genetic and probably also protein polymorphisms related to the Th1 and Th2 inflammatory pathways. We also recommend further wider studies for the estimation of cytokine levels in the serum and cultured inflammatory and immune cells of patients with T2DM. Lastly, we conclude, taking into consideration the fact that genetic polymorphisms are population specific, this study probably provides a presumptive evidence that the heterozygous *IL*-4-590 *CT* and *IL*-13-1112 *CT* genotypes could be considered risk genotypes, while the homozygous wild *IL*-4-590 *CC* and *IL*-13-1112 *CC* genotypes might be considered protective to the development of T2DM in Egyptian subjects.

Conflict of Interests

The authors declare complete freedom of any issue concerning conflict of interests related to this work.

Acknowledgment

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