Association of C-469T of Interleukin 13 Gene Polymorphism with Vitiligo Development in Thi Qar Province/South of Iraq

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Abstract

Vitiligo is a common disorder characterized by the appearance of white patches resulting from the loss of functional melanocytes and melanin from the skin. Recent studies have associated vitiligo with defective autoimmune system. The Interleukin-13 (IL-13) gene polymorphism maybe one of the important conditions for the development of a certain group of diseases, especially autoimmune diseases. The aim of this study was to investigate the association between C-469T of IL-13 gene polymorphism (Figure 1) and vitiligo in Thi Qar province in southern Iraq. This study consisted of 100 subjects including 60 vitiligo patients (40 males, 20 females, mean age 31.85 ± 15.1 years) and 40 matched controls. No significant differences were observed between the two studied groups as regards sex, age and smoking. Restriction Fragment Length Polymorphism (RFLP) PCR was used for the analysis of the studied polymorphism. The distribution of IL-13 C/T and T/T genotypes did not show significant difference between the patients and controls P-value=0.6 (OR=1.4; 95 %CI=0.53-3.75), P-value=0.59 (OR=0.76; 95 %CI=0.28-2) respectively.

Keywords: Interleukin; Polymorphism; Vitiligo; PCR-RFLP

Introduction

Vitiligo is an acquired disorder characterized by the appearance of white patches resulting from the loss of melanocytes and melanin from the skin. This disorder affects 1–4% of the world population. There are several hypothesis concerning the etiology of vitiligo have been suggested including autoimmunity, inherent defects in melanocyte biochemistry and neuronal dysfunction [1]. Among these, believes the most well accepted is the autoimmune theory, supported by findings that are proven the frequent occurrence of other autoimmune diseases in vitiligo patients and their relatives [2] also the presence of auto-antibodies and auto-reactive T-lymphocytes in vitiligo patients, both with cytotoxic effects upon melanocytes [3]. The immune system is complex, involving cell-mediated and humoral mechanisms – both of which appear to play roles in the manifestation of vitiligo. Identifying pathways involved in the immune reactions in vitiligo will help in understanding the cause of vitiligo and pave the way for developing specific immune targets to combat the disease. Co-occurrence with autoimmune diseases seems to be dependent of the clinical type of vitiligo especially with nonsegmental type [2]; however, the observation is not as frequent when considering exclusively the segmental clinical type of vitiligo [4]. The changing in cytokines levels were reported in vitiligo affected skin compared with healthy skin suggesting that the cytokine production in epidermal microenvironment may play a role in pathogenesis of vitiligo [5,6]. Several studies showed the role of peripheral blood and lesional cytokine expression in vitiligo patients. These suggested a role for epidermal cytokine imbalance in the pathogenesis of vitiligo [6,7].

Increased levels of a class of proteins called interleukins (ILs) might be linked to the active stage of vitiligo [8]. Several studies investigated the putative role of cytokines in vitiligo by studying IL-17 [9], IL-10 [7], IL-2, IL-6 [10]. IL-6 and IL-13 secreting CD8+ T cells from vitiligo perilesional margins may induce autologous melanocyte apoptosis [11]. Increased concentrations of serum IL-10, IL-13, and IL-17A and decreased concentrations of TGF-β1 suggested altered cell-mediated immunity that may facilitate the melanocyte cytotoxicity in vitiligo [8].

Human Interleukin-13 (IL-13) is a 17-KDa glycoprotein. IL-13 is produced mainly by activated Th2 cells [12] and is contributed in the maturation and differentiation of B cells [13]. The IL-13 gene is located on chromosome 5q31 in the cluster of genes encoding IL-3, IL-4, IL-5 and IL-9. It has four exons and five introns [14]. More than thirty one single nucleotide polymorphisms have been identified in the IL-13 gene of human. Most of these polymorphisms are in the 5 regulatory region or in intron (non-coding region) of the gene. The most widely studied SNPs are promoter polymorphism T-1112C and anonsynonymous
polymorphism located in exon A-2044C. Functional studies have explained the association between the IL-13 SNPs and phenotype of some diseases such as asthma [15,16], diabetes [17].

Subjects and Methods

In this study sixty patients with vitiligo disease were randomly selected. The subjects composed of 40 males and 20 females, whose ages ranged from (1–58) years. They were from clinic of dermatology of Al-Hussein Teaching Hospital in the province of Thi Qar. Forty healthy cases were studied as matched controls. All the cases studied had no other autoimmune diseases. Blood samples were collected from all subjects into an EDTA vacutainer tubes (2.5 ml). Genomic DNA was extracted from blood leukocytes using a spin column protocol [18] by gSYNCTM DNA Mini kit.

C-469T Polymorphism

The C-469T polymorphism in the IL-13 gene was detected by a polymerase chain reaction –restriction fragment length polymorphism (PCR-RFLP) method was suggested by Graves and Coworkers [19]. The following primers were used in PCR technique, F- 5-CCT AGG CAG GCA ACA TAG TG-3 and R-5-CTG GAC CCT TCT CAA TAA GT-3. PCR was carried out in a total volume of 20 μl with 5 μl of DNA, 1 μl of each primers, 5 μl master mix, 9 μl distal water. The amplification conditions were explained in Table 1.

Table 1 Program of polymerase chain reaction technique.

| No. of steps | Steps         | Temperature | Time      | No. of cycle |
|--------------|---------------|-------------|-----------|--------------|
| 1            | Initial denaturation | 94°C        | 1 min     | 1 Cycle      |
| 2            | Denaturation   | 94°C        | 40 sec    |              |
|              | Annealing     | 52°C        | 40 sec    | 35 Cycles    |
|              | Extension     | 72°C        | 50 sec    |              |

RFLP Analysis

The products of PCR were cleaved with Accl as follows: 5 μl of the PCR product were added to 1 μl of the enzyme and then 2 μl buffer and 10 μl sterile distilled water were added, followed by incubation at 37°C for 2 hours and then electrophoresed on 2% agarose stained with ethedium bromide.

Statistical Analysis

Statistical analysis of this study was conducted, using the mean, standard deviation, chi-square test and odd ratio test with 95% confidence intervals (95% CI) by SPSS V.17.

Results

Clinical features were showed in Table 2. In this study, the mean age of patient group was 31.85 ± 15.1 years and the mean of age onset was 20.9 ± 13.7. Regarding to gender, 66.67% of patients were male while 33.33% were female. 68.33% of cases were non-smokers and 31.37% were smokers. 35% of patients have family history, meanwhile the rest have no family history.

Amplification of flanking regions of C-469T resulted in 549 bp, 303 bp and 246 bp bands. The PCR-RFLP analysis for C-469T SNP generated two fragments in case of TT genotype and the fragments were 303 bp and 246 bp. As for CC genotype a 549 bp was seen. On the other hand, all three bands 549/303/246 bp could be showed in case CT genotype.

For C-469T SNP, the results showed no significant of CT genotype P=0.48 (OR=1.4; 95 %CI=0.53-3.75) in patients compared to control group (Figure 1). Also TT genotype was no significantly more frequent in patients P=0.59 (OR=0.76; 95% CI=0.28-2) (Table 3). T allele frequent did not show significant compared to C allele in patient and control groups P=0.6 (OR=0.85; 95% CI=0.48 - 1.52) (Table 4).

Table 2 Clinical features of vitiligo patients and control group; P value ≤ 0.05 significant*.

| Clinical features     | Cases (n=60) | Controls (n=40) | P value |
|-----------------------|--------------|-----------------|---------|
| Mean age (year, M ± SD) | 31.85 ± 15.1 | 30.8 ± 11.2     |         |
| Age of onset (year, M ±SD) | 20.9 ± 13.7  | —               |         |
| Gender                |              |                 | 0.93    |
| Male                  | 40           | 66.67%          | 27      | 67.5%  |
| Female                | 20           | 33.33%          | 13      | 32.5%  |
| Smoking               |              |                 |         |
| Non smoker            | 41           | 68.33%          | 30      | 75%    | 0.47   |
Table 3 The frequency of C-469T of IL-13 gene polymorphism in vitiligo patients and control group. 95% CI, Confidence Interval. OR, Odds ratio; P value ≤ 0.05 significant*

| Polymorphic Genotypes | Patients (n=60) | Control (n=40) | OR   | CI 95%   | P value |
|------------------------|----------------|---------------|------|----------|---------|
| CC                     | 26 (43.33%)    | 17 (42.5%)    | 1    | —        | —       |
| CT                     | 14 (23.33%)    | 13 (32.5%)    | 1.4  | 0.53 - 3.75 | 0.48    |
| TT                     | 20 (33.33%)    | 10 (25%)      | 0.76 | 0.28 - 2  | 0.59    |
| Total                  | 60 (100%)      | 40 (100%)     | —    | —        | —       |

Table 4 The frequency of IL-13 C and T alleles in vitiligo patients and control group. 95% CI, Confidence Interval. OR, Odds ratio; P value ≤ 0.05 significant*

| Polymorphic Genotypes | Patients (n=60) | Control (n=40) | OR   | CI 95%   | P value |
|------------------------|----------------|---------------|------|----------|---------|
| C                      | 66 (55%)       | 47 (58.75%)   | 1    | —        | —       |
| T                      | 54 (45%)       | 33 (41.25%)   | 0.85 | 0.48 - 1.52 | 0.6     |
| Total                  | 120 (100%)     | 80 (100%)     | —    | —        | —       |

Figure 1 Electrophoresis of C-469T of IL-13 gene polymorphism on agarose gel after digestion by AccI enzyme.

Discussion

No significant differences were observed between the two studied groups as regards sex, age and smoking. The mean age was 31.85 ± 15.1 years and 30.8 ± 11.2 years of cases and control group respectively. This finding is similar to Usha and Pandey [20] who found the mean age of patient and control group was 33.23 ± 16.67 and 31.45 ± 11.02 respectively. Age of onset of disease was 20.9 ± 13.7. Most of studies demonstrated the onset of vitiligo was generally at less than 30 years in age [21,22]. Vitiligo does not show any significant association with gender (P=0.10). Although the majority of patients were male (66.67%), but we found no significant in gender between the vitiligo patients and the control group (P=0.93). Other study showed that disease was more common in female [21]. We found no significant between patient and control group according to smoking (P=0.47). This finding suggested by [20,23]. A positive family history appeared in 35 % of patients. Also [23] found 30% of cases had positive family history. Less than that [24] reported 20% of cases had family history.

The autoimmune hypothesis for vitiligo was provided the basis for a vast number of experimental designs and studies. Even the IL-13 gene polymorphism is considered one of the important prerequisites for the development of a certain group of diseases, especially autoimmune diseases [25], but there is no study on the IL-13 gene polymorphism and development of vitiligo. In this paper, we have investigated polymorphisms of the C-469T of IL-13 gene to find the association between the C-469T of IL-13 gene polymorphisms and the susceptibility to vitiligo.

Although the heterozygous CT polymorphic genotype increases risk of vitiligo by once and half approximately (OR=1.4; 95% CI=0.53-3.75), the statistical analysis did not show a correlation between this genotype development of vitiligo P-value=0.6. Also the genotype distribution and allele frequency TT did not show significant difference between the patients and controls (OR=0.76 ; 95 % CI=0.28-2) P-value=0.59.

Regarding to individual alleles, the frequency of C allele was (55%) in vitiligo patients and (58.75%) in control subjects, while the frequency of T allele was higher in vitiligo patients (45%) as compared to control subjects (41%) (Table 3).

Conclusions

As we know, this study is the first insight look into the C-469T polymorphic of IL-13 gene and the susceptibility to vitiligo. It showed no association between C-469T polymorphic of IL-13 gene and development of vitiligo.

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