Lack of Association between Catalase Gene Polymorphism and Susceptibility to Vitiligo in an Egyptian Population

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Abstract

Background: Vitiligo is an acquired hypomelanotic skin disorder resulting from the loss of functional melanocytes from the cutaneous epidermis. Low catalase (CAT) activity and accumulation of hydrogen peroxide (H2O2) have been demonstrated in the epidermis of vitiligo patients. Some polymorphisms on catalase gene may have effect on the quantity and activity of catalase enzyme. The aim of this study was to investigate whether catalase (CAT) gene polymorphisms are associated with susceptibility to vitiligo in Egyptian population.

Materials and methods: Thirty patients with vitiligo and twenty gender, age and ethnic matched controls were enrolled in the study. Genotyping was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: The CAT gene -89A>T and 389C>T genotypes and allele frequencies of vitiligo patients did not differ significantly from those of healthy controls.

Conclusions: We found no association between CAT gene -89A>T and 389C>T polymorphism and vitiligo susceptibility in Egyptian vitiligo patients. Further studies with greater sample size should be performed to verify these results.

Keywords: Catalase; Gene polymorphism; Vitiligo; Pigment cell

Abbreviations

Bp: Base Pair; CAT: Catalase; 95% CI: 95% Confidence Intervals; OR: Odds Ratios; PCR: Polymerase Chain Reaction; RFLP: Restriction Fragment Length Polymorphism; SNP: Single Nucleotide Polymorphism

Introduction

Vitiligo is a common, chronic skin disorder resulting from selective destruction of melanocytes. The disease affects individuals of both sexes; with prevalence ranging from 0.1 to 8.8% worldwide [1-3]. The etiology of vitiligo is still unknown, although the disease was first noted in approximately 1500 B.C. Several theories have been proposed to explain the pathogenesis of vitiligo, including self-destructive, biochemical, neural, auto-immune and genetic hypotheses [4]. In recent years, oxidative stress has attracted much attention, and some findings have suggested that oxidative stress may be the triggering event in the melanocyte degeneration of vitiligo [5-11]. Epidemiological studies have shown that vitiligo tends to aggregate in families [12-17]. About 20% of vitiligo patients have at least one first-degree relative with vitiligo. The relative risk of developing vitiligo is increased 7 to 10 fold in first degree relatives of vitiligo patients [14]. Previous studies of vitiliginous melanocytes showed that an imbalance between oxidative and antioxidative patterns, such as the accumulation of hydrogen peroxide (H2O2) and low Catalase (CAT) level/activity, may induce the destruction of melanocytes [18-22].

Catalase is the enzyme that catalyzes the conversion of hydrogen peroxide to water and oxygen, thereby preventing cellular damage due to highly reactive oxygen-derived free radicals. The CAT gene was selected as a candidate gene because of the reduction of catalase enzyme activity and concomitant accumulation of excess hydrogen peroxide observed in the entire epidermis of vitiligo patients [18]. The cause of low CAT level/activity in vitiligo patients has not been determined. In addition to substrate inhibition of CAT activity by high H2O2 levels in the epidermis of vitiligo patients, allelic variants in the CAT gene may have deleterious effects on the expression or function of CAT [23]. The human CAT gene is located on chromosome 11p13, spanning 34 kb of genomic DNA consisting of 13 exons and 12 introns [24]. A number of CAT gene Single-Nucleotide Polymorphisms (SNPs) and mutations have been associated with disease manifestations such as catalasemia/hypocatalasemia, hypertension, and type 2 diabetes mellitus in various races [25-28]. An association
has been established between vitiligo and a Single Nucleotide Polymorphism (SNP) in exon 9 of the CAT gene [29]. It has been documented that T/C heterozygotes are more frequent among vitiligo patients than controls, and that the C allele is transmitted more frequently to patients with vitiligo compared to controls, suggesting that linked mutations in or near the CAT gene may contribute to a quantitative deficiency of catalase activity in the epidermis and accumulation of excess hydrogen peroxide [29-34]. The objective of this study was to determine a possible association between the CAT gene and vitiligo susceptibility in an Egyptian population using this marker.

Material and Methods

Study participants

This hospital-based case-control study was conducted at the department of Dermatology and Venereology, Al Azhar University, Assuit, Egypt. Thirty Egyptian vitiligo patients (5 men and 25 women) and twenty sex-, age- and matched controls (6 men and 14 women) without any clinical evidence of vitiligo were enrolled in the control group. Their ages were 38.23 ± 3.19 (mean ± SE) and 42.45 ± 3.31 respectively. Family history of vitiligo was present in only 4 patients with vitiligo. The study was approved by the local institutional ethics committee of faculty of medicine, Al-Azhar University. All participants were informed about the nature of the study, and written informed consent was obtained.

Polymorphisms and genotyping

The CAT (-89A>T and – 389 C>T) polymorphisms were determined using the PCR–restriction fragment length polymorphism method. PCR amplifications were generated using the following primers: for CAT _89A>T, forward 5'-AATCAGAAGGCAGTTCCC-3' and reverse 5'- TCGGGGAGCACAGAGTGTAC-3' (product of 250 bp); for CAT 389C>T, forward 5'-GCCGCCTTTTTGCCTACTCCCT-3' and reverse 5'-TCCCGCCCATCTGCTCCAC-3' (product of 202 bp). PCR was performed using DNA as a template under the following conditions: 94°C for 10 minutes, then 30 cycles of 94°C for 55 seconds, annealing temperature (-89A>T at 56°C, 389C>T at 58°C) for 55 seconds and 72°C for 90 seconds, and final extension at 72°C for 10 minutes. Restriction fragment length polymorphism–PCR was used to analyze _89A>T, using HinfI to digest the 250 bp PCR amplification products, which resulted in 177 and 73 bp fragments in the case of the A allele. In addition, 389CAT was analyzed by restriction fragment length polymorphism–PCR with BstXI, which cuts only the T allele, resulting in 108 and 94 bp fragments.

Statistical analysis

The data were analyzed using sigma stat (version 3.5) Differences in the alleles or genotypes frequencies were examined by χ² test. Consistency of genotype frequencies with the Hardy-Weinberg equilibrium was tested using a χ² test on a contingency table of observed vs. expected genotype frequencies in each group. P values <0.05 were considered statistically significant.

Results

Table 1: Genotype and allele frequencies of the CAT polymorphism among the cases and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (n= 30)</th>
<th>Controls (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT – 389 C&gt;T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>22</td>
<td>16</td>
<td>0.67</td>
</tr>
<tr>
<td>CT</td>
<td>7</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CAT -89 A&gt;T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>18</td>
<td>13</td>
<td>0.92</td>
</tr>
<tr>
<td>AT</td>
<td>9</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

The study included 30 vitiligo patients 5 male and 25 females their age were 8.23 ± 3.19 (mean ± SE) years and only 4 (13.3%) had family history also the types of vitiligo were 21 vulgaris, 6 focal, 1 universal and 2 acrofacial. 19 of the vitiligo patients had no symptoms, 4 had leukotrichia, 1 had diabetes, 2 had itching and 4 had Kobner phenomenon. The observed genotype frequencies of CAT -89A>T and 389C>T polymorphic sites in both the patient and control groups were consistent with the Hardy–Weinberg equilibrium. The distributions of CAT –389C>T and CAT- 89A>T genotypes among cases and controls showed no significant differences (Table 1). The relation between the alleles frequencies of CAT -389 C>T, CAT-89 A>T and the clinical characteristics of the vitiligo patients showed no significance between

Table 2: Polymorphism of CAT -389 C>T and clinical characteristics of the vitiligo patients

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Sex</th>
<th>Onset</th>
<th>Family history</th>
<th>Types</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Gradual</td>
<td>Sudden</td>
<td>Yes/Vulgaris</td>
</tr>
<tr>
<td>CC</td>
<td>5</td>
<td>17</td>
<td>19</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>CT</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>TT</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>24</td>
<td>26</td>
<td>4</td>
<td>26</td>
</tr>
</tbody>
</table>

*There is no statistically significant difference between the CAT -389 C>T and any of the clinical characteristics of the vitiligo patients
the catalase polymorphism and any of the characteristic of the vitiligo (Tables 2 and 3).

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Sex</th>
<th>Onset</th>
<th>Family history</th>
<th>Types</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Gradual</td>
<td>Sudden</td>
<td>No</td>
</tr>
<tr>
<td>CC</td>
<td>5</td>
<td>17</td>
<td>19</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>CT</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>6</td>
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<tr>
<td>TT</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>24</td>
<td>26</td>
<td>4</td>
<td>26</td>
</tr>
</tbody>
</table>

Table 3: polymorphism of CAT -389 C>T and clinical characteristics of the vitiligo patients

Discussion

Vitiligo is an acquired pigmentary disorder of unknown etiology that is clinically characterized by the development of white macules related to the selective loss of melanocytes. The prevalence of the disease is around 1% in the United States and in Europe, but ranges from less than 0.1% to greater than 8% worldwide. A recorded predominance of women may reflect their greater willingness to express concern about cosmetically relevant issues. Half of all patients develop the disease before 20 years of age [3]. Onset at an advanced age occurs but is unusual, and should raise concerns about associated diseases, such as thyroid dysfunction, rheumatoid arthritis, diabetes mellitus, and alopecia areata [4]. Generalized vitiligo is the most common clinical presentation and often involves the face and acral regions. The course of the disease is unpredictable and the response to treatment varies. Depigmentation may be the source of severe psychological distress, diminished quality of life, and increased risk of psychiatric morbidity.

Genetics of vitiligo is characterized by incomplete penetrance, multiple susceptibility loci and genetic heterogeneity [11,14]. Approaches for the identification of genes involved in vitiligo pathogenesis have taken a number of forms, initially focusing on biological candidates and differential expression analyses. In the last decade, technological advances enabled by human genome project, and methodological advances applied to the analyses of polygenic, multifactorial diseases have permitted more global approaches, and genome-wide scans. As the result, there has been considerable progress in identifying susceptibility genes for vitiligo.

Catalase is well known antioxidant enzyme, which prevents cell damage from highly Reactive Oxygen Species (ROS). The CAT gene was selected as a candidate gene because of the reduction of catalase enzyme activity during vitiligo condition and concomitant accumulation of excess hydrogen peroxide in the entire epidermis of vitiligo patients [6]. Many allelic variants of catalase have been reported and the first form was known as catalasemia, which occurred due to splicing mutation in Japanese population [6]. The CAT gene composed of 13 exons spanning 33kb of genomic DNA located on chromosome 11p13 with the complete cDNA sequence revealing a coding region 1584 base pairs in length [5]. It is known that SNP in exon 9 of the CAT gene is associated with vitiligo [5], as T/C heterozygocity is more frequent in vitiligo patients than in controls. C allele is transmitted more frequently to patients than to controls, suggesting that linked mutations in or near CAT gene may contribute to a quantitative deficiency of catalase activity in patients with vitiligo.

All these data indicate that CAT gene is an attractive therapeutic target for vitiligo [5].

The past two decades have seen reports of increased epidermal oxidative stress in the cutaneous epidermis of vitiligo patients and suggesting that increased prooxidant activities and reduced antioxidant activities can lead to elevated levels of H2O2 [28,29]. The relationships between the polymorphisms of the catalase enzyme and various diseases have been investigated [27]. The CAT has an important role in protecting cells against severe oxidative stress, and allelic variants in the CAT gene could have deleterious effects on the expression or function of CAT, which may result in high sensitivity to H2O2 [23]. Patients with vitiligo exhibit decreased catalase levels in epidermis as a result of inactivation by elevated hydrogen peroxide concentrations [17]. In addition, allelic variants in the CAT gene which have harmful effects upon the expression or function of CAT could play a part in the low levels of enzyme activity which may result with increased sensitivity to H2O2 [18].

To the best of our knowledge, there are a few studies examining the association of vitiligo and -89A>T polymorphism on the promoter region of CAT gene. First study was performed by Ling Liu et al. and they found that the -89A>T variant genotype was associated with a significantly increased risk of vitiligo in Chinese population [17]. However in the other two studies, Park et al and Akbas et al, determined no significant correlation between -89A>T polymorphism and vitiligo in Korean and Turkish populations respectively [18,19]. Similar to the results of Park et al. and Akbal et al. we found no significant association between -89A>T polymorphism and vitiligo in our selected Egyptian population. Controversial results regarding to the association of -89A>T polymorphism and vitiligo seems to be related with role of ethnicity. The 389C>T polymorphism in exon 9 of the CAT gene results in silent substitution of aspartic acid. In a Meta-analysis Lv et al. found a significant correlation between the CAT gene 389C>T polymorphism and the risk of vitiligo. On the Contrary CAT 389C>T polymorphism was associated with susceptibility to vitiligo in a North American and English population [20,21]. But, there was no difference between normal controls and vitiligo patients in Korean, Chinese population or Turkish populations [18,19]. In accordance with those Korean, Chinese and Turkish studies [18-20] our study determined that there was no difference between normal controls and vitiligo patients in terms of 389C>T polymorphism in Egyptian population.

In conclusion, our results suggesting that the CAT -89A>T and 389C>T polymorphism is not associated with vitiligo susceptibility in
Egyptian population. The low catalase activity in vitiligo patient epidermis is more likely to result from environmental conditions such as inhibitory levels of hydrogen peroxide rather than allelic variations in the catalase gene. Further studies with greater sample size should be performed to verify these results.

References


