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Angiotensin converting enzyme (ACE) gene polymorphism in vitiligo: protective and predisposing effects of genotypes in disease susceptibility and progression

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Angiotensin converting enzyme (ACE) gene polymorphism in vitiligo: protective and predisposing effects of genotypes in disease susceptibility and progression

Vitiligo is a depigmenting skin disorder with profound heterogeneity in its aetio-pathophysiology, and is associated with inter-individual variation in progression of disease. Angiotensin converting enzyme (ACE) is a regulator of renin angiotensin system (RAS) that plays an important role in the physiology of the vasculature, blood pressure, inflammation, adipocyte distribution of various diseases. The present study was carried out in 243 vitiligo patients (132 males and 111 females), aged between 3-62 years with a mean age at onset of 21.6 ± 13.6 yrs, and in 205 healthy controls of south Indian origin. The main objectives of the present study were to evaluate the ACE I/D (insertion/deletion) polymorphism in the patient and control groups. Further, I/D genotypes were compared among the patients with and without the family history of vitiligo as well as the progression of the disease, through polymerase chain reaction (PCR) methods. The results revealed a highly significant association of DD genotype with disease susceptibility (p < 0.01) in patients with a family history of vitiligo (p < 0.05) in terms of early age at onset. Further, the pre-dominance of ID genotype among patients revealed its association with a slow progression of the disease (p < 0.05). The present study is the first report to highlight the protective role of II genotype and the significant association of ID genotype with slow progression of the disease.

Key words: angiotensin converting enzyme, progression, protection, susceptibility, vitiligo


ID (insertion/deletion heterozygote) having intermediate levels of the enzyme [14, 15]. It has been reported that these genotypes exhibit variations with respect to oxidative stress, angiogenesis, vasoconstriction and distribution of subcutaneous fat [16, 17].

In view of existing literature on ACE insertion/deletion polymorphism, it was felt that ACE was a relevant polymorphic marker for oxidative stress and angiogenesis as well as body fat distribution. We aimed to investigate the role of ACE insertion/deletion polymorphism not only in susceptibility to vitiligo but also in the progression of the depigmentation process, a novel aspect which has not been dealt in other studies.

Materials and method

Our study enrolled 243 vitiligo cases from South India, which were examined in the vitiligo unit at the Central Research Institute of Unani Medicine (CRIUM, Hyderabad, India). These cases were not suffering from any other skin or autoimmune disorder. As a control group, 205 healthy age and sex matched volunteers without any clinical evidence of vitiligo or other skin disorders were recruited. This study was approved by the ethical committee of CRIUM and Department of genetics, Osmania University (Hyderabad). All subjects were included only after informed consent for clinical and demographical data was obtained. Based on the progression of the depigmentation, patients were categorized as fast or slow progressive types. If the patients showed depigmentation of more than three quarters of the total body surface area within one year of the disease manifestation, they were categorized as fast progressive type and if it was less than one quarter of the total body surface area they were categorized as slow progressive type. As there is no standard classification of the rate of progression of disease, our categorization is based on the long term observation of CRIUM dermatologists (unpublished) and from studies on treatment response in vitiligo [9-11].

ACE gene polymorphism

Blood samples were collected from patients and controls and were subjected to DNA isolation by standard procedure. ACE genotyping was carried out by polymerase chain reaction using oligonucleotide sense primer 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′, and the antisense primer 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′. DNA samples (100 ng) were subjected to 35 cycles of PCR amplification in eppendorf thermocycler under the following conditions; initial denaturation 94 °C for 5 min, denaturation 94 °C for 45 sec; annealing 58 °C for 1 min; extension 72 °C for 45 sec and final extension of 72 °C for 7 min. PCR products were analyzed with 2% agarose gel electrophoresis and ethidium bromide staining in order to identify three patterns: II (a 490 bp fragment), DD (a 190 bp fragment) and ID (both 490 and 190 bp fragments).

Statistical analyses

Statistical analysis for relative risk was done by Odds ratio with 95% confidence interval. ANOVA was carried out for association of ACE insertion/deletion polymorphism and age at onset in relation to familial history. The statistical package for social sciences (SPSS, 15th version) was used to perform the analysis. Hardy-Weinberg equilibrium was evaluated by χ² test for genotypic and allelic frequencies in the patient and control groups.

Results and discussion

We analyzed the polymorphism of ACE gene in 243 patients and 205 healthy volunteers. Based on CRIUM observation (unpublished) 50 (20.6%) patients were categorized as fast progressive and the remaining 193 (79.4%) as slow progressive types. The age at onset was 1-59 yrs of the patients and it was lower in the fast progressive (1-49yrs) compared to the slow progressive group (1-59yrs). The overall mean age at onset was 21.6 ± 13.6 yrs. However, it was found to be 22.3 ± 14.7 yrs and 21.4 ± 13.3 yrs in the fast progressive and slow progressive types, respectively. Of the 243 vitiligo patients, 54 (22.2%) individuals showed a family history of the disease.

ACE I/D polymorphism in disease susceptibility

The frequencies of ACE I/D genotypes in vitiligo patients and controls are given in table 1 and figure 1. Analysis of genotype frequencies revealed an over-representation of DD and ID among patients, compared to that of the control group (p < 0.05). This observation indicates increased susceptibility of the DD genotype to vitiligo. However, the apparent difference of the ID genotype between patients and controls was not significant.

ACE, being a pleiotropic gene, may be involved in susceptibility to vitiligo, due to its multiple effects. The role of the ACE gene is implicated in oxidative stress, angiogenesis and the distribution of body fat. Earlier reports on ACE insertion/deletion polymorphism in disease association have suggested the role of angiogenesis in vitiligo, while in some other diseases its role is suggested in enhanced reactive oxygen species (ROS) and fat distribution [16, 17]. Of the 3 ACE I/D genotypes, DD is considered to be associated with relatively enhanced ROS generation mediated by angiotensin II, compared to other genotypes [17]. Moreover, individuals with this homozygous genotype DD have also been reported to have greater accumulated visceral fat, which may be contributing to the disease manifestations associated with high oxidative stress like diabetes and cardiovascular disease [18-21]. These adverse pathophysiological effects of the DD genotype, along with other susceptible genes, may predispose individuals to a dermatological condition like vitiligo. The aetiological association of vitiligo involves not only oxidative stress but also angiogenesis, which may facilitate the access of cells of the immune system and of auto-antibodies to the site of melanocyte destruction.

Analysis of the II genotype frequency revealed an almost 50% reduction in the frequency of this genotype among the patients compared to controls. It suggests a protective role of the II homozygous condition against the development of vitiligo, as the II genotype is suggested to be less
Table 1. Distribution of ACE I/D genotypes in vitiligo patients and controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>II (%)</th>
<th>ID (%)</th>
<th>DD (%)</th>
<th>$\chi^2$ (p value)</th>
<th>Allele frequency</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>54 (22.3)</td>
<td>115 (47.3)</td>
<td>74 (30.4)</td>
<td>18.2 (0.000)</td>
<td>0.45</td>
<td>0.55</td>
</tr>
<tr>
<td>Controls</td>
<td>83 (40.5)</td>
<td>80 (39)</td>
<td>42 (20.5)</td>
<td></td>
<td>0.60</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Note: NS is not significant.

ACE I/D genotypes in relation to family history of vitiligo and influence on age at onset of the disease

Of the total 243 patients, 22.2% (n = 54) show a positive family history of vitiligo, whereas 77.8% (n = 189) were without family history of vitiligo. When the ACE I/D polymorphism was analyzed, 16.7%, 46.3% and 37% of the familial cases showed II, ID and DD genotypes respectively. However, 23.8%, 47.6% and 28.6% of non-familial cases revealed the above genotypes. When genotype vs family history of vitiligo with age at onset was analyzed by ANOVA, it was observed that the individuals with a family history and the DD genotype had an early age at onset of the disease, indicating that the DD genotype may be contributing to early age at onset of vitiligo (Fisher’s value 32.95, p < 0.01).

ACE I/D polymorphism in disease progression

In addition to disease susceptibility, another important factor to be noted is disease progression, which is defined as enlargement of the existing depigmented lesions and/or appearance of new depigmented areas. Inter-individual variations in disease progression among patients are frequently observed, warranting genetic marker association analysis that may help to predict disease progression in patients. In view of this variation, we analyzed ACE I/D polymorphism in two groups of patients, namely fast progression and slow progression types of the disease. Out of 50 cases observed in the fast progressive group, 13 (26%) showed II genotype and 18 (36%) had ID genotypes. The DD genotype was observed in 19 (38%) cases. In the slow progressive group, which comprised of 193 patients, 41 (21.2%) individuals were of II genotype, 97 (50.2%) individuals with ID genotype and the remaining 55 (28.4%) were of DD genotype. Analysis of the proportion of II and DD homozygotes in the fast progressive and slow progressive groups revealed no significant differences. However, there was an about 9.6% increase in the frequency of the DD genotype among the fast progressive group compared to the slow progressive group. Further, it was observed that the percentage of individuals with an ID genotype was significantly reduced in the fast progressive compared to the slow progressive group (36% vs 50.2%) (table 2, figure 2).

Based on our results, it is likely that disease progression may be more due to an angiogenic effect as it facilitates access of cells of the immune system, as well as auto-antibodies, to the site of melanocyte destruction. The observations made in the present study, like the substantial increase in the frequency of the DD genotype in the fast progressive group (9.6%) compared to the slow progressive group, are suggestive of angiogenic effects of the D allele in a homozygous condition. This assumption is supported by reports of a DD homozygote association with diabetic nephropathy, which
Table 2. Distribution of ACE I/D genotypes in vitiligo patients with fast progressive and slow progressive type.

<table>
<thead>
<tr>
<th>Groups</th>
<th>II (%)</th>
<th>ID (%)</th>
<th>DD (%)</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast progressive</td>
<td>13 (26)</td>
<td>18 (36)</td>
<td>19 (38)</td>
<td>I: 0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D: 0.56</td>
</tr>
<tr>
<td>Slow progressive</td>
<td>41 (21.2)</td>
<td>97 (50.2)</td>
<td>55 (28.4)</td>
<td>I: 0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D: 0.54</td>
</tr>
</tbody>
</table>

Note: NS is not significant.

is explained on the basis of enhanced neovascularization in the kidney [25, 26], progression of sarcoidosis [27] and severity of systemic lupus erythematosus [28]. Moreover, metastasis is observed in cancers more frequently in DD individuals, which is mainly attributed to angiogenic effects [29-31].

The patterns of genotype association with vitiligo susceptibility and progression are different. The DD genotype is observed to be associated with a significantly increased susceptibility (p - 0.000). Contrary to this, in disease progression we observed a 14% decrease in the frequency of ID in fast progression, indicating that a heterozygous condition slows down the disease progression. As vitiligo pathogenesis involves both oxidative stress and autoimmunity, it appears that autoimmune mechanism(s) have a decisive role in progression, while in disease susceptibility oxidative stress mechanisms play a relatively more important role.

Converse to our expectation, the ID genotype was associated with a reduced risk of disease progression compared to the other two genotypes (p < 0.05). It appears that an as yet not understood mechanism of allelic interaction is associated with this pleiotropic gene; the oxidative stress-inducing role of D allele predominates in the susceptibility and the angiogenic role in disease progression. However, II in the homozygous condition is more prominent in a protective role. Though these two alleles are co-dominant in their expression, their product interaction seems to be complex. It is suggested that, in order to understand the role of ACE (a pleiotropic marker on susceptibility and progression of disease), certain markers of angiogenicity and adipocyte distribution could be studied with respect to vitiligo in different populations.

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References