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Full Length Research Paper

Association of angiotensin converting enzyme gene I/D polymorphism with vitiligo in South Indian population

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Vitiligo or leukoderma is a chronic skin condition that causes loss of pigment due to destruction of melanocytes, resulting in irregular pale patches of skin. Vitiligo is polygenic disease and associated with autoimmunity. Angiotensin converting enzyme (ACE) is capable of modulating cutaneous neuro-genic inflammation. An insertion/deletion (I/D) polymorphism of a 287-base pair repetitive sequence in intron 16 of the ACE gene was reported to have been associated with autoimmunity and with the development of vitiligo. In our study, the distribution of ACE gene I/D genotype was investigated in a population of 186 South Indian vitiligo patients and 201 healthy controls using polymerase chain reaction genotyping method. The ACE genotype and allele frequency (^{2} = 9.576, P= 0.008) (^{2} = 10.68, P=0.001) were significantly different between vitiligo patients and healthy controls.However there was no significant difference between the segmental and non-segmental vitiligo(^{2} = 0.182, P = 0.91) detected in ACE gene genotype distribution .This study suggests that the ACE genepolymorphism confers susceptibility to vitiligo.

Key words: Angiotensin converting enzyme, gene polymorphism, vitiligo, autoimmunity.

INTRODUCTION

Vitiligo is an acquired depigmentary disorder character-rized by the appearance of white patches resulting from the loss of functional melanocytes, Mollet I et.al (Jul 2007) and melanin from the skin and is associated with autoimmunity, Le Poole and Luiten (2008). Vitiligo affects 1 - 4% of the world's population1, Ortonne and Bose (1993); irrespective of gender and race. Its prevalence is varying from 0.46 to 8.8% in India, Handa and Kaur (1999). The Gujarat and Rajasthan states have the high-est prevalence that is, around 8.8% Valia and Dutta (1996) .Age at onset is variable and theaverage age of onset is about 20 years, Groysmane and Sami (2008). The etiology of the disease remains unknown and several hypotheses exist to explain the etiology. Autocytotoxic (self-destruct) hypothesis is based on the preferential destruction of melanocytes by toxic intermediates of melanin synthesis, while neural hypothesis suggests that the melanocytes could be damaged by neurochemical mediators released from the nerve endings supported by the association of vitiligo with neurological disorders or with peripheral nerve injury, Kovacs (1998). The inheri-tance of vitiligo may involve genes associated with the biosynthesis of melanin, a response to oxidative stress, and regulation of autoimmunity, Halder and Taliaferro (2008). The genetics of vitiligo cannot be explained by simple Mendelian genetics, Zhanq and JUN-Chen (2005) it is characterized by incomplete penetrance, multiple susceptibility loci and genetic heterogeneity, Spritz (2008). A few genes that are reported to contribute to viti-ligo susceptibility are Autoimmune regulator I Gene (AIRE I), Cytotoxic T lymphocyte antigen 4 (CTLA 4), Catalase (CAT), Catechol-o-methyl transferase (COMT), Light molecular weight protein (LMP) , transporter asso-ciated protein (TAP), Angiotensin converting enzyme gene (ACE), Melanocortin 1 receptor (MC1R) , agouti signaling protein (ASIP) genes and Lymphoid protein tyrosine phosphatase (PTPN 22) gene—PTPN, Shajil et al (2006). The Angiotensin converting enzyme (ACE) gene was selected as a candidate gene as it plays an important role in the physiology of the vasculature, blood pressure and inflammation, and its relationship with various diseases, including autoimmune diseases, has

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Table 1. Base line demographics.

Vitiligo (n = 186)			
	Non-segmental n = 109	**Segmental n=77**	**Controls n=201**
Average age (yr, Mean ± SD)	30±12.2	29.8 ± 12.2	29 ± 9.8
Male / Female (N)	66/43	48/29	125/76
Onset age (yr, Mean ± SD)	22±12.1	25.5 ± 12.2	
Smokers	28	18	10
Alcoholic	21	17	4
Duration of Disease (yr, Mean ±SD)	8±7.7	4.3±4	

has been widely investigated. The I/D polymorphism of ACE gene in vitiligo patients has been reported. Angiotensin converting enzyme (ACE) is a potent vaso-constrictor of the renin–angiotensin system, and it also inactivates bradykinin, a vasodilator of the kal ikrein–kinin system, which has major implications in the inflammatory process, Viita nen (2001).A study showed that ACE is capable of modulating cutaneous neurogenic inflammation, Scholzen T(2003).Furthermore, the association between ACE I/D polymorphism in intron 16 and autoimmune disease was reported, Papadopoulos et al (2000). The association of ace gene I/D polymorphism with vitiligo and autoimmunity had varied results from different ethnic population. In this study, the distribution of ACE gene I/D genotypes was investigated in a population of South Indian patients with vitiligo and age, sex and ethnical y matched controls.

MATERIALS AND METHOD

A total of 186 South Indian patients (114 men and 72 women) were examined at the Dermatology Clinic at Central Research Institute of Unani Medicine and diagnosed as vitiligo, not suffering from any other skin or autoimmune disorder and 201 healthy control subjects (125 men and 76 women) who presented no clinical evidence of vitiligo or any other skin and autoimmune disorder were included in the study. This study was approved by the ethics review of Central Research Institute of Unani Medicine (CRIUM) committee and all subjects gave informed consent. The 186 south Indian vitiligo patients (114 male, 72 female; mean age: 30 years; age range: 9 - 69 years; mean age at disease onset: 24 years; age range at disease onset: 1– 66years) were characterized with segmental vitiligo 77 and non-segmental 109. The 201 ethnically matched healthy controls (125male, 76 female; mean age: 29 years; age range: 11 – 61 years) had no clinical evidence or family history of vitiligo or of any other autoimmune disorder.

ACE gene polymorphism analysis: Genomic DNA was prepared from heparinized venous blood samples using salting out method with slight modification. The sense oligonucleotide primer was 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', and the antisense primer was 5'-GAT GTG GCC ATC ACA TTC GTC AGA T- 3' .DNA samples (100 ng) were subjected to 30 cycles of PCR amplification in Biorad System under the following conditions; initial denaturation 94^{O}C for 5 min, denaturation 94^{O}C for 1 min; annealing 58^{O}C for 1min; extension 72^{O}C for 1 min; and 72^{O}C for 7 min . PCR products were analyzed with 2% agarose gel electrophoresis and ethidium bromide staining in order to identify three patterns: I/I (a 490 bp fragment), D/D (a 190 bp fragment) and I/D (both 490and 190 bp fragments).

Statistical analyses

The frequencies of the I/D alleles and genotypes were compared between vitiligo patient and control groups using a ^{2} test on 2 × 2 and 2 × 3 contingency tables. Statistical significance was accepted at $P < 0.05$. Odds ratios (OR); 95% Cl were computed to assess the strength of the association between the presence of the ACE gene polymorphism and vitiligo.

RESULTS

The baseline demographic characters of the patients and controls were given in Table 1. The mean age of onset of non-segmental disease group was found to be at a younger age than that of segmental vitiligo. Non-segmental group were found to have chronic vitiligo when compared with segmental group.

The genotypic distribution of ACE gene polymorphism and allelic frequency for I and D alleles in patients and controls have been give in Table 2. It was observed that patients showed a comparatively much higher percentage of the DD genotype indicating homozygous mutant 32%, compared to 23% of II genotype. The ID genotype was found at a rate of about 45% among patients. Among the control, the rate of occurrence of the II genotype with a frequency of 37%, while the ID and DD genotypes were found to be 42% and 21% respectively.

The ACE gene genotype distribution (P = 0.008) and alele frequency (P = 0.001) were significantly different between vitiligo patients and healthy controls. In addition,

Table 2. Genotyping of ACE gene polymorphism

Genotype	Patients	Control	^{2}	P-Value
II	44(23)	74(37)		
ID	83(45)	84(42)	9.576[a]	0.008
DD	59(32)	43(21)		
Allelic frequencies				
I	171(46)	232(58)	10.68 [b]	0.001
D	201(54)	170(42)		

a. Controls vs patients using the chi-square test with 3 × 2 contingency table.
b. Controls vs patients using the chi-square test with 2 × 2 contingency table Odds ratio of ID, DD and D allele were 0.60 (95% CI; 0.36-1.00), 0.44 (95% CI; 0.24-0.77) and 0.610 (95 % CI; 0.5-0.81) respectively.

Table 3. Genotyping of subgroup of vitiligo.

Genotype	Non-Segmental	Segmental	$_2$	P-Value
II	27(31)	17(22)		
ID	48 (25)	35(45)	0.182	0.91
DD	34 (44)	25(33)		
Alleles				0.07
I	102 (47)	69(45)		
D	116 (53)	85(55)	0.14	

Odd ratio of non segmental and segmental genotype ID OR = 1.16 (95% CI 0.51-2.62) DD OR = 1.17 (0.49 – 2. 80) D allele OR = 1.08 (0.70- 1.67).

the results indicated that the D allele was significantly over -represented in the patients with vitiligo compared with controls (54% Vs 42%). However no statistical y significance variation (P = 0.91) was observed in the genotype with in the case groups and the results were tabulated in Table 3.

DISCUSSION

Considered the most common pigmentary disorder, vitiligo involves complex interaction of environmental and genetic factors that ultimately contribute to melanocyte destruction, resulting in the characteristic depigmented lesions. In the past few years, studies of the genetic epidemiology of vitiligo have led to the recognition that generalized vitiligo is part of a broader autoimmune disease diathesis. Attempts to identify genes involved in susceptibility to generalized vitiligo have involved gene expression studies, genetic association studies of candidate genes, and genome-wide linkage analyses to discover new genes. Vitiligo is suggested to have an autoimmune etiology, although this remains undefined.

The frequent association of vitiligo with autoimmune disorders, Ochi and DeGroot (1969), the demonstration of autoantibodies to melanosomal proteins in the serum of patients with the disease, Baharav et al. (1996), Cui et al. (1995), Kemp et al. (1997), Song et al. (1994) and evidence that vitiligo autoantibodies can destroy pigment cells *in vitro*, Norris (1998) and *in vivo* ,Gilhar et al. (1995) support this theory. Experiments in murine models have also demonstrated that CTLs to melanocytic antigens can cause the destruction of pigment cells leading to vitiligo-like depigmentation, Overwijk et al. (1999). These observations provide evidence of T cell - mediated vitiligo in both humans and mice and suggest that unregulated T cell activity may contribute to the development of the disease. Several genes that have a role in regulating immunity have been associated with susceptibility to vitiligo. Vitiligo can also develop as part of autoimmune polyendocrine syndrome type 1, a disease resulting from mutations in the autoimmune regulator (AIRE) gene, Nagamine et al. (1997). Previous investigations have shown that elevated levels of ACE have been associated with autoimmune disease, Czernobilsky et al. (1985) and an insertion/deletion (I/D) polymorphism of a 287-base pair (bp) repetitive sequence in intron 16 of the ACE gene accounts for a large proportion of the variability of serum ACE activity with DD genotypes having the highest and II genotypes having the lowest ACE, Rigat et al. (1992) thus ACE confers susceptibility to autoimmune disorders and vitiligo, Papadopoulos et al. (2000).

Although the pathogenic mechanism has not been established in either case, ACE and its related substrates or products are known to have various functions in the immune system, Kozlowski et al. (1992) and in the inflammatory response. There are few indications that

ACE may involve vitiligo development. ACE is also capable of degrading the substance P (SP) and other peptide mediators. In the skin, neuropeptides such as SP are released from sensory nerves in response to noxious stimuli like chemical and mechanical injury, Scholzen et al. (1998, 2003). SP can induce or augment inflammatory responses such as plasma extravasation, leukocyte activation, cytokine production,and mast cel activation.

Other possible mechanism includes that ACE gene expression in melanocytes may be different between patients and control. In the present study of 186 patients with vitiligo; we found that the polymorphic variant D allele was associated with the disease. Here association was found between ace genotype and allele frequencies for the I/D polymorphism and vitiligo in south Indian population (^{2} = 9.576, P = 0.008) (^{2} = 10.68, P = 0.001) suggesting that ACE confers susceptibility to vitiligo. Our findings indicate potential interactions between the ACE and vitiligo and can reproduces a variety of clinical manifestations, and may contribute to identifying the underlying autoimmune pathophysiology of vitiligo.

These results were similar to earlier findings that demonstrated a significant difference in allelic frequencies between vitiligo patients without other autoimmune disease and normal controls, Papadopoulos et al. (2000) but were dissimilar to that of Gujrat population, Mitesh D. et al. (2008) and also the English population, Akhtar et al (2005). The difference may reflect differences in the geographic location and of the industrial area of the subjects under study. We observed that non-segmental vitiligo had early age of onset and longer duration of the disease when compared to segmental type. However there was no significant difference in the ace genotype (^{2} = 0.182, P = 0.9) or allelic frequencies (^{2}= 0.14, P = 0.07) between the two groups, segmental and non-segmental, of vitiligo patients.

Conclusion

This kind of a statistically significant variation in the frequency of insertion/deletion polymorphism in both populations strongly indicates that ACE gene polymorphism confers susceptibility to vitiligo in the south Indian population. Our results also suggest that the DD geno-types at the ACE gene locus might be an important gene-tic risk factor for vitiligo thus supporting its autoimmune etiology.

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