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Distribution of angiotensin converting enzyme gene polymorphism among Northern Hans, Daurs, and Ewenkis

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KEY WORDS peptidyl-dipeptidase A; genes; polymorphism (genetics); polymerase chain reaction

ABSTRACT

AIM: To observe the polymorphism of angiotensin-converting enzyme (ACE) gene in Northern Hans, Dahurs, and Ewenkis of China. **METHODS**: The polymerase chain reaction was used to type the insertion/deletion polymorphism at intron 16 of ACE gene among 90 Northern Hans, 84 Dahurs, and 64 Ewenkis individuals. The experiment displayed the distribution in three kinds of ACE genotype: ID (heterozygotes of insertion and deletion), DD (homozygotes of deletion), and II (homozytes of insertion). **RESULTS**: In Northern Hans the percentages of the distributing ACE I/D genotype were ID 27.8 %, DD 17.8 %, and II 54.4 %. The I/D genotype frequency of Dahurs individuals were ID 60.7 %, DD 26.2 %, and II 13.1 %. The Ewenkis genotype frequency were ID 70.3 %, DD 21.9 %, and II 7.8 %. **CONCLUSION**: The polymorphism of ACE gene of Northern Hans is different from that of Dahurs and Ewenkis in China.

INTRODUCTION

The functions of the angiotensin-converting enzyme (ACE) include the metabolism of bradykinin and the conversion of angiotensin Ⅰ to angiotensin Ⅱ. A strong interfamilial resemblance of serum ACE together with segregation studies suggest that a major gene regulates the circulation level[1]. The human angiotensin-1 converting enzyme gene covers 21 kilobase pairs (kb) and comprised 26 exons and 25 introns. The ACE has been assigned to 17q23. The ACE gene consists of 300-bp Alu sequence within intron 16, and I/D polymorphism is observable[2]. The ACE gene I/D polymorphism may have important clinical relevance. A number of associations of the polymorphism in cardiovascular disease have now been recognized[3,4], which may be related to the higher level of ACE that accompanies the presence of the D allele. The relationship of ACE gene polymorphism was described in a study that includes white and black subjects. In the present study, there is no literature if the polymorphism has differences in different minorities. The report discovered the polymorphism distribution of ACE gene in three disparate minorites. It can provide data for human genetic research, and clarify the incidence of diseases of some illness in three minorities from level of gene. There are many minorities in Bilingual League of Inner Mongolia Autonomous Region, and the present study reported the polymorphism distribution of ACE gene in three minorities.

MATERIALS AND METHODS

Materials A sample ($n = 90$) of Hans subjects was a healthy population, Dahurs ($n = 84$) and Ewenk samples ($n = 64$) were randomly taken from the healthy population in Xilinguole League of Inner Mongolia Autonomous Region. We collected 5 mL venous blood to distill DNA.

Methods Genomic DNA was isolated from the blood leucocytes by standard proteinase K-phenol method. The PCR assay for I/D polymorphism was performed as literature described[2]. Briefly, 1 μL genomic DNA amplified with a forward primer (5'CTG GAG ACC ACT CCC ATC CTT TCT 3'), and a reverse primer (5' GAT GTG ATC ACA TTC GTC AGA T 3'). Amplification was performed in a final volume of 10 μL, contained 7.5 pmol/μL each primer (Huamei company, Beijing), 1 μL $MgCl_2$, 1 μL KCl, 1 μL of each dNTP, and 0.3 μ Taq polymerase (Huamei company, Beijing).

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DNA amplification was achieved by an initial denaturation at 92 ℃ for 120 s, denaturation at 92 ℃ for 30 s, anneal at 58 ℃ for 45 s, and extension at 72 ℃ for 60 s, and total 32 cycles, then final extension at 72 ℃ for 600 s. PCR products were subjected to 2 % agarose gel electrophoresis, and two alleles were identified: a 190-bp fragment D (in the absence of the deletion) and a 490-bp fragment I (in the presence of the insertion).

Satistical analysis Significant difference in frequency distribution was assessed using chi-square test. P less than 0.05 is considered to be statistical significance.

RESULTS

The PCR genotype of I/D polymorphism of the ACE gene (Fig 1). The Pgem 100-bp DNA marker (Promega USA) is shown at right. The 490-bp insertion fragment denotes the I allele, the 190-bp deletion fragment indicates the D allele, the 490-bp and 190-bp fragment are insertion/deletion heterocyclin fragment. Namely, II, DD, ID three genotype. The distribution of II, ID, and DD genotype in three ethnic groups are shown in Tab 1. The genotype frequency distribution in Northern Hans group were ID 27.8 %, DD 17.8 %, II 54.4 %. Dahurs populations were ID 60.7 %, DD 26.2 %, II 13.1 %. Ewenkis populations were ID 70.3 %, DD 21.9 %, II 7.8 %. In contrast, the genotype frequency of Hans have marked difference with Dahurs and Ewenkis ($\chi^2 = 56.59$, $P < 0.05$). There is no difference in the genotype frequency between the Dahurs and Ewenkis groups ($\chi^2 = 1.48$, $P > 0.05$).

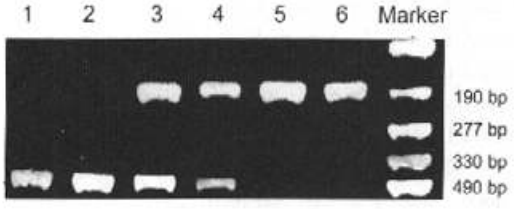

Fig 1. PCR genotyping of I/D polymorphism of ACE gene. The 490-bp fragments denotes the I allele, and the 190-bp fragment indicates the D allele. Lane 1 and 2: genotype II, Lane 3 and 4: genotype ID, Lane 5 and 6: genotype DD.

DISCUSSION

The functions of the angiotensin-converting enzyme covered the metabolism of bradykinin and converted angiotensin I to angiotensin II[5]. Angiotensin II is an octapeptide that has vasoactive and sodium-retaining activities along with acapacity to stimulate vascular proliferation[6]. We have shown that frequency of the I allele of the ACE gene was similar to the values reported in the Japanese, but relatively higher in normal Northern Hans, compared with white[7] and black populations in Europe, African-Americans[8], and Africans[9], however lower than in Samoans[10] (Tab 2). The polymorphism of ACE gene in different ethnic groups of the same region was not reported. We found that the Northern Hans showed marked difference compared with Dahurs and Ewenkis. There is highest II genotype in Hans, but there is highest I D genotype in Dahurs and Ewenkis. This difference might be related to genetic background.

The results of the former study of white people confirmed ACE gene I/D polymorphism associated with serum ACE[11]. The level of ACE activity was significantly higher in the white people with D alleles than that with I alleles, whereas the level of ACE activity was intermediate in those who were heterozygous. On the other hand, in black no association of the I/D polymorphism with serum ACE activity was found. There was thus a distinctly different association of the ACE gene polymorphism with the regulation of serum ACE activity in whites and blacks. Rigat's research indicates marked difference between the serum ACE levels which observed and subjects in each of the three ACE genotype classes[11]: Serum immunoreactive ACE concentrations were 299.3, 393, 494 mg/L for II, ID, DD genotype respectively. The insertion/deletion polymorphism accounted for 47 % of total phenotypic variance of serum ACE. As for Northern Hans, Dahurs, Ewenkis, it merits further research the relation between the polymorphism and the serum ACE.

Recent work on the I/D ACE polymorphism has demonstrated that the DD genotype is associated with increased risk of cardiovascular diseases, especially in those without other risk factors[12], increased death rate and sudden death of ischemia heart disease, hytrophic cardiomyopathy[12-15]. Meantime, positive associations have been observed between the ACE D allele and essential hypertension, myocardial infarction, and left ventricular hypertrophy[16,17]. Clinical trails have also shown the benefits of ACE inhibitors in hypertension and congestive heart failure and decreased the risk of reinfarction in patients who experience acute myocardial infarction[18].

Tab 1. *Genotypic and allelic frequencies of I/D polymorphism of the ACE gene among three nationalities.*

Nationality	*n*	Genotype frequency			Allele type frequency	
		II	ID	DD	D	I
Hans	90	49 (54.4 %)	25 (27.8 %)	16 (17.8 %)	32.2 %	67.8 %
Dahur	84	11 (13.1 %)	51 (60.7 %)	22 (26.2 %)	57.0 %	43.0 %
Ewenk	64	5 (7.8 %)	45 (70.3 %)	14 (21.9 %)	57.8 %	42.2 %

Tab 2. Frequency of I/D polymorphism of ACE gene in different groups.

Ethnic Group	*n*	Genotype frequency			Reference
		II	ID	DD	
Japanese	76	33.00 %	49.00 %	18.00 %	2
British	98	17.00 %	43.00 %	40.00 %	3
American-Whites	139	21.58 %	47.40 %	30.94 %	7
American-Blacks	223	12.00 %	51.00 %	37.00 %	8
Nigerian	80	16.00 %	49.00 %	35.00 %	9
Samoans	58	82.76 %	15.52 %	1.72 %	10

In conclusion, this article indicates that the polymorphism of ACE gene of Hans differs from that of Dahurs and Ewenkis of China. From the point of view of anthropology, the polymorphism has important significance to acknowledge the different clinical representation of hypertension, coronary heart disease and diabetes in different races. With regard to appraising ACE inhibitor clinic exertion and curative effect, and the relevancy which the difference of the polymorphism of ACE gene and curative effect of ACE inhibitor are not neglectful factor, and that they deserve to further discuss.

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血管紧张素转换酶基因多态性在北方汉族、达斡尔族、鄂温克族中的分布

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关键词 肽基-二肽酶 A；基因；多态现象(遗传学)；聚合酶链反应

目的：观察血管紧张素转换酶(ACE)基因多态性在北方汉族、达斡尔族和鄂温克族中的分布. **方法**：采用聚合酶链反应(PCR)检测 90 例北方汉族，84 例达斡尔族和 64 例鄂温克族 ACE 基因内含子 16 的插入/缺失多态标记. 得到三种基因型：插入/缺失杂合子(ID)、缺失纯合子(DD)、插入纯合子(II). **结果**：北方汉族 ACE 基因频率 ID 27.8 %、DD 17.8、II 54.4 %. 达斡尔族 ID 60.7 %、DD 26.2 %、II 13.1 %；鄂温克族 ID 70.3 %、DD 21.9 %、II 7.8 %. **结论**：中国北方汉族与达斡尔族和鄂温克族间 ACE 基因多态性存在差异.

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