Original Article

Apolipoprotein E Gene Polymorphism in the Mongolian Population

(apolipoprotein E / genetic polymorphism / Mongolia / population study / ε4 allele)

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Abstract. Apolipoprotein E plays a key role in the regulation of lipid metabolism. ApoE function is determined by the presence of three common alleles (ε2, ε3, ε4). The *apo* ε3 allele is the most prevalent, apo ε2 is associated with dysbetalipoproteinaemia, and *apo* ε4 is frequently associated with an increased risk for cardiovascular and Alzheimer's diseases. Mongolian population has a high rate of cardiovascular mortality and morbidity and there might be genetic susceptibility of the population to cardiovascular disease. The aim of our study was to establish the frequency of *apoE* genotypes in 744 Mongolian subjects and to compare the results with findings from other Asian populations. The apo E sequence was amplified using polymerase chain reaction and apo E genotyping was performed by restriction enzyme cleavage with *Cfo*I. The relative *apoE* allele frequencies were ε2 = 3.7%, ε3 = 80.8%, and ε4 = 15.5%, the genotype frequencies were ε2/ε2 = 0% (N = 0), ε2/ε3 = 5.7% (N = 42), ε2/ε4 = 1.7% (N = 13), ε3/ε3 = 65.3% (N = 486), ε3/ε4 = 25.4% (N = 189), ε4/ε4 = 1.9% (N = 14); the occurrence of the risk ε4 allele in Mongolia is among the highest in Asia. The high frequency of the apo ε4 allele may increase the susceptibility of Mongolian population to cardiovascular diseases.

Introduction

Apolipoprotein (apo) E plays a crucial role in the metabolism of plasma lipoproteins. ApoE is one of the major protein constituents of several lipoprotein classes and serves as a ligand for the low density lipoprotein (LDL) receptor and LDL receptor-related protein (LRP), thus removing ApoE-rich lipoproteins from the plasma. It is also involved in cholesterol absorption from the intestine and in reparative and remodelling processes in the central nervous system (Mahley, 1988; Huang et al., 2004).

The gene coding for ApoE is located on the long arm of chromosome 19. A polymorphism in the 4th exon of the *apoE* gene determines the three common alleles (ε2, ε3, ε4) in human population coding for three common isoforms of apo E (E2, E3, E4); the ε3 allele is the most frequent, with prevalence of 70–80% in most populations. The isoforms differ from each other by amino acid substitution at positions 112 and 158. The presence of three alleles leads to the formation of six different phenotypes: E2/2, E2/3, E2/4, E3/3, E3/4, E4/4 (Utermann et al., 1977; Hatters et al., 2006).

The isoforms differ in their receptor binding affinity. ApoE3 and E4 bind to receptors with similarly high affinity, whereas ApoE2 shows less than 2% of the normal binding affinity. However, the association of ApoE isoforms with plasma lipid levels and with clinical diseases is not straightforward. Despite its low receptor binding affinity, ApoE2 is usually associated with lower total and LDL cholesterol levels, while in E2 homozygotes, it is often associated with dysbetalipoproteinaemia. Relation of ApoE2 to the risk of atherosclerosis is controversial. ApoE4 is associated with decreased longevity, increased plasma total and LDL cholesterol and ApoB levels and increased prevalence of cardiovascular disease (CVD) and also of Alzheimer's disease (Smith, 2000; Hatters et al., 2006).

The distribution of the three alleles varies across populations, which may have clinical implications. In particular, the differences in the occurrence of the ε4 allele may contribute to the regional variation in the risk of cardiovascular and Alzheimer's diseases (Siest et al., 1995).

Mongolia is a developing country with high prevalence of CVD, and cardiovascular risk factors are also

Received February 19, 2007. Accepted March 19, 2007.

This project was supported by the Ministry of Education, Youth and Sports, Czech Republic, research project 0021620807.

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Abbreviations: apo – apolipoprotein, CVD – cardiovascular disease, PCR – polymerase chain reaction.

increasing as a result of industrialization and lifestyle changes. Under these circumstances, the distribution of *apoE* alleles can influence cardiovascular risk of the Mongolian population (Manaseki, 1993; Neupert, 1995). We therefore studied the *apoE* gene polymorphism in the Mongolian population.

Material and Methods

Apo E genotype was examined in the total number of 744 unrelated healthy volunteers from various regions of Mongolia; 621 subjects (275 women, 346 men; age 37.6 ± 14.3 years) were recruited in three large cities (Ulaanbaatar, Darchan, Erdenet) and 123 subjects (63 women, 60 men; age 39.5 year ± 16.5 years) were from six rural areas in different parts of Mongolia.

Capillary blood for DNA analysis was collected on FTA matrix cards (Whatman, BioScience, Cambridge, UK). These cards are specially designed for the collection, archiving, purification and analysis of DNA. When specimens are spotted onto the card, cell membranes and organelles are disintegrated, and the nucleic acids are entrapped in the fibres of the card matrix.

For DNA analysis, a 2-mm disk was punched from within the middle of the dried blood stain. Using FTA Purification Reagent (Whatman), bound nucleic acid was purified by washing out the haem and other components that would inhibit polymerase chain reaction (PCR) and restriction enzyme reaction. Nucleic acids remained immobilized within the matrix during purification. The washed disk was then transferred to the PCR reaction tube; the DNA content was released out only during the PCR.

The *apoE* genotype was determined by restriction analysis using *Cfo*I following partial amplification of exon 4 of the *apoE* gene using PCR. PCR was conducted in a thermal cycler (BIO-RAD, My Cycler, Hercules, CA). The oligonucleotide primers used for amplification were P1 (5′-TCCAAGGAGCTGCAGGCGGCGCA-3′) and P2 (5′-ACAGAATTCGCCCCGGCCTGGTACACTGCCA-3′) (Wenham et al., 1991). The amplification mixture contained 2 mM $MgCl_2$, 1.5 unit of FastStart Taq DNA Polymerase in the 1x GC-rich solution and buffer provided by the manufacturer (Roche, Basel, Switzerland), 0.2 mM of each dNTP (Promega, Madison, WI), 0.8 μM of each primer and 2-mm disk with DNA in a final volume of 49.5 μl. The PCR conditions were initial denaturation at 95°C for 4 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing and elongation at 67.3°C for 1 min 30 s, respectively. A final extension step at 72°C for 10 min was done. The amplification generated a DNA fragment of 227 bp.

After amplification, 21 μl of the PCR product were directly digested with 12 units of the restriction endonuclease *Cfo*I (Promega) at 37°C overnight (Reymer et al., 1995). Gene fragments were separated using 10 % vertical polyacrylamide gel electrophoresis and detected by ethidium bromide staining under ultraviolet illumination, using an appropriate DNA size marker.

To compare frequencies of *apoE* alleles in the Mongolian population with those in other Asian countries, we searched the Medline database for papers reporting on *apoE* data from this area. All available data were considered; we included data from population-based studies and from various groups of healthy subjects; data from patient cohorts with a particular disease were excluded. For each country, allele frequencies were calculated using the weighted average.

The allelic and genotypic frequencies of *apoE* were estimated by counting alleles and genotypes and calculating sample proportions; the statistical significance of differences of frequencies between groups was compared by χ^2 test. The distribution of *apoE* polymorphism was tested for Hardy-Weinberg equilibrium using χ^2 goodness-of-fit test.

Results

The overall frequencies of *apo* ε2, ε3, and ε4 alleles are shown in Table 1. There were no significant differences between the urban and rural areas; we also did not observe any differences between men and women in our study.

The frequencies of *apoE* genotypes are presented in Table 2. The observed distribution was compared to the expected frequencies by χ^2 test, the genotype frequencies did not differ from Hardy-Weinberg equilibrium ($df = 3$, $P > 0.05$).

Table 3 shows the allelic frequencies of *apoE* in several Asian countries; the frequency of the risk *apo* ε4 allele in the Mongolian population is the highest among the countries. Data for the Buryats (one of the Mongolian ethnic groups) are from a small study in an isolated area of eastern Mongolia.

Table 1. The frequencies of apo ε2, ε3, and ε4 alleles in 744 subjects from urban and rural areas of Mongolia

Allele	Allele frequency (%)		
	Total (N = 744)	Urban population (N = 621)	Rural population (N = 123)
ε2	3.7	3.9	2.4
ε3	80.8	80.4	83.3
ε4	15.5	15.7	14.2

Table 2. The frequencies of individual apoE genotypes in 744 Mongolian subjects; check for Hardy-Weinberg equilibrium (df = 3, P > 0.05)

Genotype	Observed frequency		Expected frequency		$\chi 2$
	N	%	N	%	
ε2/ε2	0	0	1.02	0.14	1.02
ε2/ε3	42	5.65	44.49	5.98	0.14
ε2/ε4	13	1.75	8.53	1.15	2.34
ε3/ε3	486	65.32	485.73	65.29	0.00
ε3/ε4	189	25.40	186.36	25.05	0.04
ε4/ε4	14	1.88	17.88	2.40	0.84
Total	**744**	**100**	**744**	**100**	**4.38**

Table 3. The allelic frequencies of apoE in several Asian countries including our study; the populations are listed according to the occurrence of ε4 allele in the ascending order

Population	Allele frequency (%)			References
	ε2	ε3	ε4	
India	4.0	88.3	7.6	1, 2, 3, 4
China	7.9	84.1	7.8	1, 2, 5, 6, 7, 8
Taiwan	1.7	89.7	8.7	9
Japan	4.4	85.6	10.0	10, 11, 12
Korea	8.2	80.6	11.2	13, 14
Mongolia	**3.7**	**80.8**	**15.5**	
Buryats	3.2	80.4	16.4	15

References: (1) Tan et al., 2003; (2) Hallman et al., 1991; (3) Thelma et al., 2001; (4) Singh et al., 2001; (5) Kobori et al., 1988; (6) Kao et al., 1995; (7) Zhang et al., 1999; (8) Jin et al., 2004; (9) Hsieh et al., 2002; (10) Nakayama and Kuzuhara, 1999; (11) Tsukamoto et al., 1993; (12) Eto et al., 1986; (13) Kima et al., 1999; (14) Kim et al., 2001; (15) Tsunoda et al., 2002.

Discussion

We determined *apoE* genotypes in 744 subjects from Mongolia. Our study is the first one performed on a large random sample of the Mongolian population. The genotype frequencies were in Hardy-Weinberg equilibrium. We found very high occurrence of the *apo* ε4 allele which may increase susceptibility of the Mongolian population to cardiovascular disease.

Representative data from Mongolia are missing; until now, only two small studies from Mongolia have been published. Our study is the first reporting population data on *apoE* allelic frequencies in Mongolia, including comparison between urban and rural areas. The frequencies we obtained correspond well with the published data from the above-mentioned study in Buryats, the population from the isolated area of eastern Mongolia (Chen, 1990; Tsunoda et al., 2002).

The distribution of *apoE* alleles varies across populations. In general, the Asian populations traditionally have lower *apo* ε4 frequency than Europeans. The studies confirmed heterogeneity of *apo* ε4 distribution in the European as well as in the Asian populations. The cause for this regional variability is still not clear. Notably, the frequency of ε4 appears to be higher in northern regions of Europe than in southern regions (Gerdes et al., 1992; Schiele et al., 2000), thus following the incidence of CVD. In Asia, a similar trend has not been described. Mongolia shows the highest frequency of *apo* ε4 allele (Table 3), while e.g. India is a country with very low ε4 allele frequency. Comparison of our results with the geographically neighbouring countries is difficult because of the lack of data from these populations. In China, the frequency of the *apo* ε4 allele is low. There are no reliable data on *apoE* polymorphism from other surrounding countries such as Kazakhstan and the Russian Siberia.

From the clinical viewpoint, the differences of ε4 allele frequencies may be important because of its association with the risk of cardiovascular and Alzheimer's diseases (Morrow et al., 2002; Greenow et al., 2005). The occurrence of the *apo* ε4 allele may therefore contribute to the variation in the risk of these diseases across populations. Analysis of allele distributions among European populations, with remarkable differences in cor-

onary artery disease (CAD) prevalence, revealed a constant positive relationship between the *apo* ε4 allele frequency and CAD incidence (Corbo et al., 1999). Mongolia is a developing country with a trend of increasing incidence of cardiovascular diseases, which is probably due to the improving living standard and westernization of lifestyle (Swinburn, 2002). The adverse effects of lifestyle factors can interact with genetic factors (e.g. *apo* ε4), thus increasing both individual and population cardiovascular risk (Komatsu et al., 2004; Ordovas, 2006). Under these circumstances, the high prevalence of ε4 can negatively influence the risk profile of the Mongolian population. Therefore, our finding of high prevalence of the *apo* ε4 allele among Mongols is not only of scientific value, but also of practical importance.

In conclusion, we examined the genotype and allele frequencies of apolipoprotein E gene in the Mongolian population. The frequency of the *apo* ε4 allele was found the highest among the other Asian populations. Since *apo* ε4 polymorphism is associated with increased risk of atherosclerosis, our findings suggest a genetic predisposition of Mongolian population to the cardiovascular disease.

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