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Population distributions of allele frequency of apolipoprotein E by age and gender in Han Chinese[1]

ZHANG Jian-Gang[2,4], DONG Xiu-Zhen[3], YANG Wei-Jun[2], LU Qing[2], HE Lin[2,4,5]
([2] *Section of Human Genome Project, Shanghai Research Center of Life Sciences, Chinese Academy of Sciences, Shanghai 200031*; [3] *Changning Dementia Hospital, Shanghai 201103*; [4] *Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS apolipoproteins E; polymorphism (genetics); ethnic groups; restriction fragment length polymorphism; DNA; polymerase chain reaction; population surveillance

ABSTRACT

AIM: To study apolipoproteins E (ApoE) allele frequency in Han Chinese based on age and gender from Shanghai metropolitan area. **METHODS**: Healthy Han Chinese people (F: 237 and M: 412) were involved in this study. ApoE gene was amplified by PCR using the forward primer: 5′-GGC ACG GCT GTC CAA GGA GCT-3′ and reverse primer: 5′-GAT GGC GCT GAG GCC GCG CT-3′. The PCR product was digested directly with 5 units of *Cfo*I and separated by a 20 % polyacrylamide nondenaturing gel. **RESULTS**: ApoE * 3 was the commonest allele which accounted for 86.4 % of the isoforms, and ApoE * 2 and ApoE * 4 accounted for 6.2 % and 7.5 %, respectively. The allele and genotype frequencies were in Hardy-Weinberg equilibrium by comparison with that of the corresponding theoretical distribution ($P > 0.05$). **CONCLUSION**: The frequencies of ApoE * 2, ApoE * 3, and ApoE * 4 were demonstrated in the normal Chinese population.

INTRODUCTION

Apolipoprotein E (ApoE) plays a central role in lipid metabolism. It is the product of a single gene on chromosome 19 with $M_r \approx 34$ kDa glycoprotein which is composed of 299 amino acid residues[1].

The ApoE allele frequencies have been found to be associated with some kind of diseases. Three common alleles of ApoE are isoforms ApoE * 2, ApoE * 3, and ApoE * 4, which can be distinguished by the variation of positions at 112 and 158 in the receptor-binding region of ApoE[2]. If the positions were with 112cys and 158arg, it formed isoform ApoE * 3 which is the commonest isoform. If the positions were with 112arg and 158arg, it formed isoform ApoE * 4 which is associated with Alzheimer's disease[3], vascular dementia[4], cognition in the very old[5], coronary artery disease[6], gallstones[7], diabetes and atherosclerosis[8], colon cancer[9]. If the positions were with 112cys and 158cys, it formed isoform ApoE * 2 which is a risk factor for type III hyperlipidemia[10] or a factor for longevity[11]. Nine populations were divided into two main groups in the light of ApoE allele frequencies[12].

These effects of ApoE genotypes on human diseases and understanding of the evolution and the origin of human beings have led to increase interests in genotyping of ApoE isoforms in population.

In this paper, ApoE allele and genotype frequencies in Han Chinese were studied by

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[5] Correspondence to Prof HE Lin. Phn 86-21-6471-7740. Fax 86-21-6433-3084. E-mail du@iris.shlc.ac.cn
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polymerase chain reaction (PCR) with restriction fragment length polymorphism analysis (RFLP).

MATERIALS AND METHODS

Subjects Peripheral vein blood sample 1 mL was collected from 649 healthy people (F: 237 and M: 412, Tab 1), respectively, whose ages ranged from 20 to 86 during May 1997 to Oct 1997. All the individuals were unrelated Han Chinese living in Shanghai region on the time when samples were collected. The subjects were selected to meet the following criteria: good physical and mental health confirmed by clinical, radiological, biological examinations, and a brief cognitive test. All blood samples collected were agreed by the donors with informed consent of ethics committee of the hospitals. The sample selection was randomized. Normally, 1 mL of each sample was collected on the arm vein, and there was no fixed time for sample collection.

Tab 1. Distributions of the subjects by age and gender. $\bar{x} \pm s$.

Age/a	Female		Male	
	n	Age/a	n	Age/a
20 - 29	19	22.1 ± 1.8	33	24.3 ± 2.5
30 - 39	43	34.7 ± 2.7	40	35.2 ± 2.3
40 - 49	44	45.3 ± 2.4	47	44.3 ± 2.8
50 - 59	12	56.2 ± 2.0	76	53.1 ± 1.9
60 - 69	31	62.6 ± 2.3	54	65.4 ± 2.2
70 - 79	75	73.8 ± 2.6	104	75.1 ± 2.2
≥80	13	86.5 ± 3.2	58	84.7 ± 3.6

Solutions PCR buffer contained: KCl 50 mmol·L^{-1}, Tris-HCl 10 mmol·L^{-1} (pH 8.3), $MgCl_2$ 2.5 mmol·L^{-1}, gelatin 0.1 g·L^{-1}, NP40 0.45 %, Tween 20 0.45 %, which was autoclaved and stored at −20 ℃. Lysis buffer contained: sucrose 0.32 mol·L^{-1}, Tris-HCl 10 mmol·L^{-1} (pH 7.5), $MgCl_2$ 5 mmol·L^{-1}, Triton X-100 1 %. Just before use, 0.6 μL of proteinase K (in H_2O) 10 g·L^{-1} was added into per 100 μL of the lysis buffer. A 10 × PCR reaction buffer contained: Tris-HCl 670 mmol·L^{-1} (pH 8.8), $(NH_4)_2SO_4$ 166 mmol·L^{-1}, $MgCl_2$ 67 mmol·L^{-1}, and β-mercaptoethanol 0.72 % (vol/vol) (Sigma).

Isolation of genomic DNA from peripheral blood The protocol was the same except some minor modifications[13]. Briefly, anticoagulated peripheral blood 100 μL was mixed with lysis buffer 0.8 mL in a 1.5 mL Eppendorf microcentrifuge tube and centrifuged at 13 000 × g for 0.5 min. The pellet was resuspended using vortex mixer in 0.8 mL of lysis buffer to centrifuge at 13 000 × g for 0.5 min. The pellet was resuspended in PCR buffer 50 μL and incubated at 60 ℃ for 1.5 h, and then incubated at 95 ℃ for another 15 min to inactivate the proteinase K. The solution was ready for PCR amplification as a template directly.

Amplification of ApoE sequences from genomic DNA Amplification of ApoE sequences of the regions encoding common ApoE isoforms from genomic DNA was conducted in a DNA Thermal Cycler (Perkin Elmer Cetus 9600) using oligonucleotide primers (forward primer: 5′-GGC ACG GCT GTC CAA GGA GCT-3′ and reverse primer: 5′-GAT GGC GCT GAG GCC GCG CT-3′). Each amplification reaction contained 100 ng of genomic DNA, 8 pmol of each primer, 5 μL of 10 × PCR reaction buffer, 10 % (vol/vol) dimethylsulfoxide (Sigma), 200 μmol·L^{-1} of dATP, dTTP, dCTP, and dGTP, 1 unit of Taq polymerase in a final volume of 50 μL. Each reaction mixture was heated at 94 ℃ for 2 min for denaturation, and subjected to 5 cycles of amplification by primer annealing (67 ℃ for 10 s), extension (72 ℃ for 80 s), and denaturation (94 ℃ for 10 s). Right after completing this mini-cycle, the reaction mixture was continuously subjected to 30 cycles of amplification by primer annealing (60 ℃ for 5 s), extension (72 ℃ for 80 s), and denaturation (94 ℃ for 10 s). The length of PCR product

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E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

was 262 bp.

Analysis of amplified ApoE product with RFLP After PCR amplification, 12 μL of each unpurified PCR product was digested with 5 units of *Cfo*I at 37 ℃ overnight. The digested product was loaded onto a 20 % nondenatured polyacrylamide gel (acrylamide : bisacrylamide = 29 : 1) with 1 mm thick × 7 cm long for 2.5 h running under 150 V. After electrophoresis, the gel was treated with ethidium bromide (0.5 mg·L^{-1}) for 6 min and DNA fragments were visualized by UV illumination. The sizes of *Cfo*I fragments were determined by the marker (*Msp*I -digested pUC18 DNA)[14].

Statistical methods Allele frequencies were calculated by allele counting. Hardy-Weinberg equilibrium was tested by a χ^2 goodness of fit test[15].

RESULTS

The distributions of ApoE * 2, ApoE * 3, and ApoE * 4 allele frequencies were 6.3 %, 85.9 %, and 7.8 %, respectively in male Han Chinese; 5.9 %, 87.1 %, and 7.0 %, respectively in female Han Chinese; and 6.2 %, 86.4 %, and 7.5 %, respectively in the total Han Chinese. (Tab 2)

Tab 2. Distributions of ApoE allele frequencies according to age and gender (%).

Age/a	ApoE * 2			ApoE * 3			ApoE * 4		
	F	M	T	F	M	T	F	M	T
20 - 29	2.6	12.1	8.7	89.5	78.8	82.7	7.9	9.1	8.7
30 - 39	10.5	8.8	9.6	86.0	78.8	82.5	3.5	12.5	7.8
40 - 49	2.3	4.3	3.3	89.8	86.2	87.9	8.0	9.6	8.8
50 - 59	8.3	5.9	6.2	87.5	88.2	88.1	3.2	5.9	5.7
60 - 69	3.2	6.5	5.3	93.5	84.3	87.6	4.2	9.3	7.1
70 - 79	6.7	5.3	5.9	84.0	88.0	86.3	9.3	6.7	7.8
≥80	7.7	5.2	5.6	80.8	89.7	88.0	11.5	5.2	6.3
Total	5.9	6.3	6.2	87.1	85.9	86.4	7.0	7.8	7.5

The ApoE * 2 allele frequency in the male group ranged from 4.3 % (age 40 - 49 a) to 12.1 % (age 20 - 29 a) and that in the female group varied from 2.3 % (age 40 - 49 a) to 10.5 % (age 30 - 39 a). (Tab 2)

The ApoE * 3 allele frequency in the male group ranged from 78.8 % (both age 20 - 29 a and age 30 - 39 a) to 89.7 % (age ≥80 a) and that in the female group varied from 80.3 % (age ≥80 a) to 93.5 % (age 60 - 69 a). (Tab 2)

The ApoE * 4 allele frequency in the male group ranged from 5.2 % (age ≥ 80 a) to 12.5 % (age 30 - 39 a) and that in the female group ranged from 3.2 % (age 50 - 59 a) to 11.5 % (age≥80 a). (Tab 2)

The allele and genotype frequencies were in Hardy-Weinberg equilibrium by comparison with that of the corresponding theoretical distribution ($P > 0.05$, Tab 3).

Tab 3. Check for Hardy-Weinberg equilibrium among 649 individuals.
$\chi^2 = 3.13$. $\upsilon = 5$. [a]$P > 0.05$ *vs* expected.

Genotype ApoE	Observed[a]		Expected		χ^2
	n	%	*n*	%	
2/2	5	0.77	2.465	0.38	2.61
2/3	64	9.9	69.09	10.65	0.38
2/4	6	0.92	5.978	0.92	0.00
3/3	486	74.9	484.1	74.59	0.01
3/4	85	13.1	83.77	12.91	0.02
4/4	3	0.46	3.624	0.5585	0.11

DISCUSSION

Although ApoE polymorphism in Western populations and ApoE allele frequencies, which are highly heterogeneous among the populations studied, in particular the relative proportion of ApoE * 2 and ApoE * 4, have been well investigated, little publication in international journals on the study of ApoE polymorphism in Han Chinese population, the largest population in the world, living in the mainland of China can be found. This should be the first report on the detailed studies of ApoE allele and genotype frequencies in Han Chinese population.

Our results are basically in agreement with Hallman *et al*. Both the Chinese and Japanese populations are with higher frequency of ApoE * 3 allele and lower frequency of the ApoE * 4, which is different from the results obtained in other populations. The frequency of ApoE * 2 allele here (0.062) is somewhat lower than that reported by Hallman (0.097) although both studies were carried out in Chinese population. These different results may be caused by the different sources of the blood samples.

The frequency of the ApoE * 4 allele here (0.075) is the same as that reported by Hallman (0.075) in the study of Chinese population.

This study shows an evidence of the frequencies of ApoE * 2, ApoE * 3 and ApoE * 4 in the normal healthy Chinese population, which can be used as reference for the further study of risk factors for relevant disease.

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中国汉族人不同年龄和性别载脂蛋白 E 等位基因频率的群体分布[1]

张建刚[2,4]，董秀珍[3]，杨伟军[2]，陆 晴[2]，贺 林[2,4,5]（[2]中国科学院上海生命科学研究中心人类基因组计划组，上海 200031；[3]上海长宁区老年痴呆专科医院，上海 201103；[4]中国科学院上海生理研究所，上海 200031，中国）

关键词 载脂蛋白 E；多态性(遗传学)；人种群；限制性内切酶片段长度的多态性；DNA；多聚酶链反应；人群监控

目的：研究在上海地区健康汉族人中不同年龄和性别的载脂蛋白 E 基因频率的群体分布。**方法**：本次调查包括 649 名健康汉族人(女：237 人，男：412 人)。载脂蛋白 E 基因用 PCR 扩增，前引物：5′-GGC ACG GCT GTC CAA GGA GCT-3′；后引物：5′-GAT GGC GCT GAG GCC GCG CT-3′。PCR 产物用 5 单位限制性内切酶 *Cfo*I 酶切，用 20 % 聚丙烯酰胺凝胶电泳分离。**结果**：ApoE * 3 频率最高为 86.4 %，其次 ApoE * 4 频率为 7.5 %，ApoE * 2 频率最低为 6.2 %。经 χ^2 检验，等位基因及基因型频率分布符合 Hardy-Weinberg 平衡。**结论**：本项研究提供了正常中国汉族人群中载脂蛋白 E 三个等位基因 2，3 和 4 频率的数据。

(责任编辑 杨如华)