RESEARCH NOTE

Is *APOE ε3* a favourable factor for the longevity: an association study in Chinese population

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Introduction

Longevity is a complex trait that is influenced by multiple genetic and nongenetic factors. Although many researchers have recently found several candidate genes that influence human lifespan, only the role of *APOE* in longevity has been consistently demonstrated. Studies indicate that the *ε4* allele, which is associated with cardiovascular disease (CVD) and Alzheimer's disease (AD), is less frequent in long-lived individuals, whereas the *ε2* allele is significantly more frequent in long-lived individuals (Smith 2002). However, we found (Y. Ze, Z. Chenguang, L. Zeping, W. Yong, W. Chen, Q. Yanchun, S. Liang, Y. Silei, S. Xiaohong, W. Li, H. Caiyou, unpublished data) that *ε3* allele frequency is significantly higher and *ε4* allele frequency is significantly lower in long-lived individuals compared to younger controls in Bama, China. Further, our data indicate that the higher frequency of *ε3* allele in long-lived individuals is due to the frequency of *ε3*/*ε3* homozygote. These findings conflict with those of previous reports. The allelic frequency of the *APOE* gene varies among populations around the world (Hallman *et al.* 1991; Singh *et al.* 2006). Therefore, whether variation in allelic frequency is responsible for the effect of the *ε3* allele on longevity is still uncertain.

Based on these considerations, we hypothesized that *APOE ε3* likely plays a significant role in longevity. Thus, after completing our study in Bama, we chose to study another independent, healthy, long-lived population in Yongfu, China. Yongfu is located 700 km east of Bama, in the same province. We applied a case–control design to confirm our previous study results.

Materials and methods

Subjects

Families were investigated in 2008 in Yongfu through personal interviews conducted by our trained staff. In addition, brief physical and biochemical examination of long-lived individuals and their offspring were carried out. A total of 1060 subjects were involved in this study. The long-lived individuals included 226 women and 93 men (mean age, 93.69 years; range, 90–108 years). Their offspring consisted of 86 women and 149 men (mean age, 42.14 years; range, 17–60 years). The healthy, local control group was composed of 506 subjects (mean age, 52.98 years; 234 women). Healthy, local controls were chosen from individuals who visited outpatient clinics in a local hospital for an annual health checkup. In the control group, individuals whose parents lived for more than 90 years of age were excluded from this study. To observe the change of allele and genotype frequency with age increment, we divided the 825 subjects composed of long-lived individuals and healthy, local controls into different subgroups by age. The age of each subject was defined by the date of birth shown on the individual's identification card or permanent resident registration card. The study protocol was approved by the ethics committee of Beijing hospital, Beijing, China. A written informed consent was obtained from all individuals recruited in this study.

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APOE genotyping

For genotyping, genomic DNA was extracted from peripheral blood leucocytes using the Wizard® Genomic DNA Purification kit (Promega, Beijing, China). Two primers (Hixson and Vernier 1990) were used for two common SNPs, c.388T>C and c.526C>T (GenBank reference sequence NM_000041.2), which produced three classic *APOE* alleles. The reaction mixture (20 *u*L) contained 50 ng of genomic DNA, 2 μM of each primer, 0.4 mM of each dNTP, 1 mM of $MgCl_2$, 10× polymerase buffers, 10% dimethyl sulphoxide (DMSO) and 1 U of DNA polymerase (Fermentas, Beijing, China). The amplification protocol consisted of initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 65°C for 30 s, and extension at 72°C for 20 s, with a final extension at 72°C for 7 min. Genotyping of SNPs was analysed by restriction fragment length polymorphism (RFLP). The PCR products were digested directly (20 μL) with 5 U of the restriction enzyme *Hha*I (Fermentas) at 37°C for 8 h. To confirm the accuracy of genotyping, we subjected DNA samples to direct sequencing.

Statistical methods

The chi-square test or Fisher's exact test was used to compare the genotype and allele frequencies between long-lived individuals and their offspring or long-lived individuals and local, healthy controls and to estimate odds ratio (OR) and 95% confidence interval (CI). We compared each genotype or allele with all other genotypes or alleles pooled together. The genotypic distribution of the long-lived individuals, their offspring and local controls was tested for the Hardy–Weinberg equilibrium (HWE) using the chi-square test. All statistical analyses were conducted by the SPSS 11.5 software package. All *P* values are two-tailed. Unless indicated otherwise, significance threshold was set at $P < 0.05$.

Results

The genotyping results by RFLP and direct sequencing are shown in figures 1 and 2 in electronic supplementary material at http://www.ias.ac.in/jgenet/. The distribution of *APOE* genotypes in long-lived individuals, their offspring and local controls were consistent with the HWE (long-lived subjects: Pearson $\chi^2 = 1.859$, d.f. = 3, $P = 0.602$; their offspring: Pearson $\chi^2 = 0.030$, d.f. = 3, $P = 0.999$; control subjects: Pearson $\chi^2 = 5.875$, d.f. = 3, $P = 0.118$). As shown in table 1, no significant difference was observed between long-lived individuals and their offspring for the distribution of the *APOE* genotype or allele frequency ($P > 0.05$). In addition, we observed the relative frequencies of *APOE* genotypes and alleles between healthy, long-lived individuals and local controls in Yongfu (table 1). The frequency of the *ε2* allele was not different among long-lived individuals and control subjects (6.7% versus 9.5%, $P = 0.05$), whereas the higher frequency of the *ε3* allele in long-lived individuals was statistically significant compared to control groups (90.4% versus 79.3%, $P = 3.02E-09$). Besides, our results show that the *ε4* allele was significantly less frequent in long-lived individuals compared to controls (2.8% versus 11.1%, $P = 1.41E-09$). In long-lived individuals compared to local controls, the homozygous *ε3/ε3* genotype was found at a higher frequency (82.4% versus 64.8%, $P = 4.53E-08$), whereas the *ε3/ε4* and *ε4/ε4* genotypes were found at lower frequencies (4.4% versus 14.0%, $P = 9.13E-06$ and 0.3% versus 3.0%, $P = 7.17E-03$).

Our findings suggest that the *ε3* allele is positively associated with longevity (OR, 2.46; 95% CI: 1.82–3.34). Further, our data show that the positive association of the *ε3* allele with longevity is the consequence of the higher frequency of the homozygous *ε3/ε3* genotype in long-lived individuals compared to local controls (82.4% versus 64.8%; OR, 2.55; 95% CI: 1.81–3.59). In contrast, the *ε4* allele was negatively associated with longevity and confers a significantly

Table 1. The distribution of *APOE* allele and genotype frequencies between long-lived individuals and their offspring or between long-lived individuals and local controls in Yongfu.

Genotype	Long-lived individual % ($n = 319$)	Offspring % ($n = 235$)	*P*	OR (95% CI)	Long-lived individual % ($n = 319$)	Local control % ($n = 506$)	*P*	OR (95% CI)
ε2/ε2	0.6	0.4	1.00	1.48 (0.13–16.38)	0.6	0.8	1.00	0.79 (0.14–4.35)
ε2/ε3	11.6	12.8	0.68	0.90 (0.54–1.50)	11.6	15.0	0.16	0.74 (0.49–1.13)
ε2/ε4	0.6	0.9	1.00	0.73 (0.10–5.26)	0.6	2.6	0.06	0.26 (0.06–1.17)
ε3/ε3	82.4	77.9	0.18	1.33 (0.88–2.04)	82.4	64.8	*4.53E-08*	*2.55 (1.81–3.59)*
ε3/ε4	4.4	7.7	0.16	0.60 (0.29–1.23)	4.4	14.0	*9.13E-06*	*0.28 (0.16–0.51)*
ε4/ε4	0.3	0.4	1.00	0.74 (0.05–11.83)	0.3	2.8	*7.17E-03*	*0.10 (0.01–0.78)*
Allele								
ε2	6.7	7.2	0.75	0.93 (0.58–1.48)	6.7	9.5	0.05	0.69 (0.47–1.00)
ε3	90.4	88.1	0.21	1.27 (0.87–1.88)	90.4	79.3	*3.02E-09*	*2.46 (1.82–3.34)*
ε4	2.8	4.7	0.14	0.62 (0.33–1.17)	2.8	11.1	*1.41E-09*	*0.23 (0.14–0.39)*

Italics denotes $P < 0.05$; *n*, number of individuals genotyped; %, relative frequency; OR, odds ratio; 95% CI, 95% confidence interval.

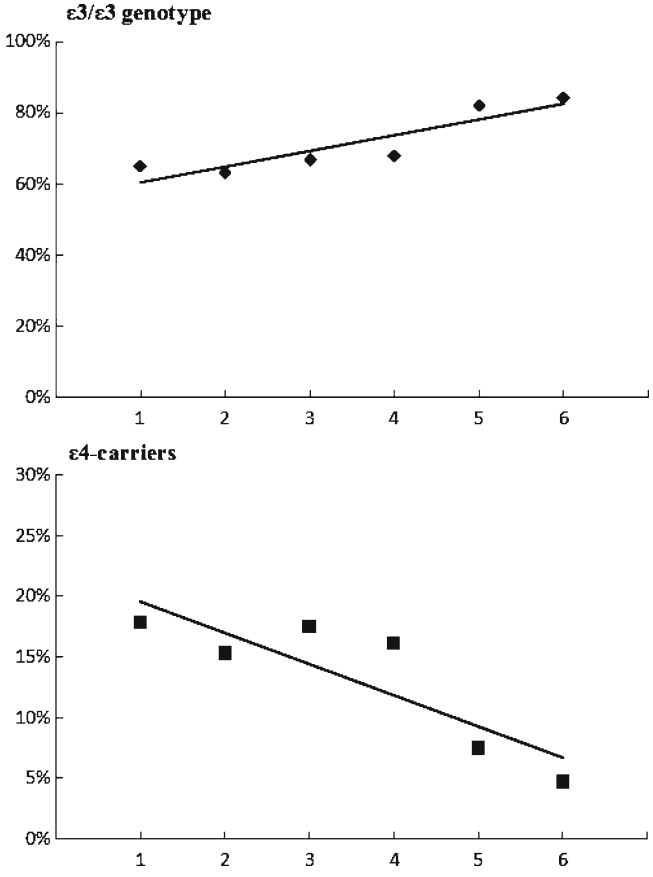

Figure 1. *APOE* genotype frequencies among different age groups in Yongfu (group 1, 40–49 years; group 2, 50–59 years; group 3, 60–69 years; group 4, 70–79 years; group 5, 90–97 years; group 6, 98 years).

increased risk for disease (OR, 0.23; 95% CI: 0.14–0.39). In addition, our findings indicate that a significant increase in *ε3/ε3* genotype frequency was found with age increment among different subgroups, whereas the frequency of the *ε4*-carrier genotypes (*ε3/ε4* and *ε4/ε4*) was decreased among older age subgroups (figure 1).

Discussion

The *ε3* allele is the most common *APOE* allele in all populations, and is generally considered as a neutral allele derived from the ancestral allele *ε4*, which increases the risk for common diseases such as CVD and AD. However, it was reported that some derived alleles that protect against common diseases can become advantageous (Di Rienzo and Hudson 2005). The *ε3* allele may have evolved adaptively and could have become advantageous with changes in environment and lifestyle. Our studies showed that the *ε3* allele was more frequent in long-lived individuals compared to local, younger controls, and the *ε3* allele was positively associated with longevity, suggesting that this allele favours longevity. Our results are different from those reported in previous studies, which we explain as follows.

Based on the Chinese census in 2000, the overall frequency of individuals ≥90 years old in China was $7/10^5$, but in Yongfu was $75/10^5$, which is approximately 10 times higher. Thus, populations in Yongfu are ideal for studies on longevity. In our studies, no significant difference was found between long-lived individuals and their offspring for the *APOE* allele and genotype frequencies, suggesting that the *APOE* gene was conserved and heritable in these families. Further, we found that the *ε3* allele was more frequent in elderly individuals and their offspring than in controls, and that this was predominantly due to the higher frequency of the homozygous *ε3/ε3* genotype but not the heterozygous *ε2/ε3* or *ε3/ε4* genotypes. Thus, we postulated that the higher frequency of *APOE ε3/ε3* genotype may be the consequence of few opportunities for gene flow of local populations, and that the homozygous *ε3/ε3* genotype is the major longevity factor in Yongfu. In addition, we assume that the *ε3* allele was selected in populations with successful ageing in Yongfu. Individuals with the *ε3* allele may have lower susceptibility to age-related diseases. This hypothesis is consistent with results showing that there is a statistically significant difference in *APOE ε3* allele frequency between nonagenarian and younger subjects in Uighur populations of China (Wang *et al.* 2001). However, the mechanism by which the *ε3* allele promotes longevity is still unknown.

Natural environments and healthy lifestyles are additional factors that may interact with the *ε3/ε3* genotype and eventually increase desirable effects of this genotype on longevity. Further study of these factors is important to obtain a clearer relationship of *APOE* polymorphisms with a long-lived individual's lifestyle as well as with other local environmental factors.

Finally, the *APOE ε3* allele was used as a reference in previous studies on the relationship of the *APOE* gene to diseases such as CVD or AD. This is because *APOE ε3* was thought to be a neutral gene with normal function. However, the positive association between the *APOE* gene and longevity, which is a healthy phenotype, makes the *APOE ε3* allele unsuitable for use as a reference. If we used *APOE ε3* as a reference, the favourable effect of *APOE ε3* on a healthy phenotype could be obscured. By comparing each genotype or allele with all other genotypes or alleles pooled together, we found *APOE ε3* was positively associated with longevity. However, this positive association was not found when *APOE ε3* was used as a reference (see table 1 in electronic supplementary material).

Previous studies showed that the frequency of the *APOE ε4* allele was significantly decreased in centenarians, whereas the *ε2* allele was significantly more frequent in long-lived individuals. In our current study, we did not find such an association of *ε2* allele with longevity. It is possible that we did not find this association because the *APOE* allelic frequencies are extremely variable in ethnic groups. Although increased frequencies of the *ε2* allele were found in long-lived individuals in European populations (Frisoni *et al.* 2001; Rea *et al.* 2001), this trend did not attain

statistical significance in Japanese, Korean or Chinese populations in previous studies (Asada *et al.* 1996; Jian-Gang *et al.* 1998; Choi *et al.* 2003). A recent large longitudinal study found increased CVD risk in male *ε2* carriers compared to *ε3*/*ε3* subjects (Pilote *et al.* 2007). In addition, several studies have indicated that *ε2* carriers have higher levels of circulating triglycerides because of defective binding affinity and less efficient formation and removal of VLDLs and chylomicrons. Therefore, the effects of the *ε2* allele on longevity are still uncertain.

In our replication study in Yongfu, we found that the *ε4* allele is less frequent in long-lived individuals compared to local younger adults. This is consistent with our previous results. In fact, the *ε4* allele seems to play an important role in successful ageing due to its inverse association with decreased longevity. Several studies have indicated that the *ε4* allele is associated with decreased longevity and increased risk for Alzheimer's and cardiovascular disease. A meta-analysis including 121 studies showed that the *ε4* allele is a significant risk factor for CVD, coupled with higher plasma cholesterol levels (Bennet *et al.* 2007). In addition, a meta-analysis of *APOE* polymorphisms and AD concluded that the *ε4* allele is associated with a significantly higher risk of AD (Bertram *et al.* 2007). Studies of the proposed mechanisms for the effects of the *APOE ε4* allele on AD and cognitive decline found that *ε4* is associated with less Aβ cellular uptake, leading to more Aβ aggregation compared to *ε3* (Smith 2002). Moreover, the *APOE ε4* allele has been associated with other pathologic conditions, including breast cancer, diabetes mellitus and diabetes-accelerated atherosclerosis, nephropathy and retinopathy (Zunarelli *et al.* 2000; Ang *et al.* 2008). Therefore, it was postulated that the ancestral *APOE ε4* allele may have been a 'thrifty' allele that subsequently became deleterious under changing environmental conditions.

In addition, we found that the increase in frequency of the *ε3*/*ε3* genotype is age-dependent, consistent with its protective effect on longevity. In contrast, the frequency of *ε4* carriers (*ε3*/*ε4* and *ε4*/*ε4* genotypes) decreased with increasing age in our data, suggesting that the disappearance of the *ε4* allele may contribute to lifespan extension. However, a follow-up study on the association between *APOE* polymorphisms and longevity indicated no effect of *APOE* genotypes on survival or mortality in very old individuals (Louhija *et al.* 2001). Therefore, we inferred that the effect of *APOE* polymorphisms on longevity may be moderate, and a number of environmental factors could modify the effects of the *APOE* gene on lifespan over a long life.

Conclusion

The distribution of the *APOE* allele and genotype in individuals with exceptional longevity appeared similar to that of their offspring. The *ε3* allele frequency was significantly higher and the frequency of the *ε4* allele was less frequent in individuals with exceptional longevity, indicating that presence of *ε3* allele or absence of *ε4* allele favours longevity. Further study involving larger sample sizes in Chinese populations is needed to confirm our findings and to further explore this hypothesis.

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