Analysis of apolipoprotein E genetic polymorphism in a large ethnic Hakka population in southern China

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Abstract

There is currently no data about the genetic variations of \textit{APOE} in Hakka population in China. The aim of this study was to analyze the allelic and genotypic frequencies of \textit{APOE} gene polymorphisms in a large ethnic Hakka population in southern China. The \textit{APOE} genes of 6,907 subjects were genotyped by the gene chip platform. The allele and genotype frequencies were analyzed. Results showed that the e3 allele had the greatest frequency (0.804) followed by e2 (0.102), and e4 (0.094), while genotype e3/e3 accounted for 65.43% followed by e2/e3 (15.85%), e3/e4 (14.13%), e2/e4 (3.01%), e4/e4 (0.84%), and e2/e2 (0.74%) in all subjects. The frequencies of the e4 allele in Chinese populations were lower than Mongolian and Javanese, while the frequencies of the e2 allele were higher and e4 allele lower than Japanese, Koreans, and Iranian compared with the geographically neighboring countries. The frequencies of e2 and e4 alleles in Hakka population were similar to the Vietnamese, Chinese-Shanghai, Chinese-Kunming Han and Chinese-Northeast, and French. The frequency of e2 in Hakka population was higher than Chinese-Dehong Dai and Chinese-Jinangsu Han. The low frequency of the \textit{APOE} e4 allele may suggest a low genetic risk of Hakka population for cardiovascular disease, Alzheimer’s disease, and other diseases.

Keywords: Apolipoprotein E, genetic polymorphism, Hakka, southern China, genotyping.

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Introduction

Apolipoprotein E (ApoE) is a multifunctional protein that plays an important role in lipoprotein metabolism, and is involved in the metabolism of very low density lipoproteins (VLDL) and chylomicrons (Blum, 2016). There are three major isoforms of human ApoE including E2 (OMIM 107741.0001), E3 (OMIM 107741.0015), and E4 (OMIM 107741.0016), as identified by isoelectric focusing. The gene coding for ApoE is \textit{APOE} (OMIM 107741), which is located on chromosome 19 in band 19q13.32 (Mahley, 1988; Siest et al., 1995). The polymorphisms in the fourth exon of \textit{APOE} gene determine three common alleles (e2, e3 and e4) coding for three major isoforms of ApoE (Martin et al., 2000; Kantarci et al., 2004; Kumar et al., 2017).

The E2, E3, and E4 isoforms differ in amino acid sequence at two sites, residue 112 (called site A) and residue 158 (called site B). At sites A/B, ApoE2, ApoE3, and ApoE4 contain cysteine/cysteine, cysteine/arginine, and arginine/arginine, respectively, which are encoded by e2, e3, and e4, respectively (Weisgraber et al., 1981; Rall Jr et al., 1982a). By different combinations of these three alleles, six genotypes (e2/e2, e2/e3, e2/e4, e3/e3, e3/e4, and e4/e4) are formed (Svobodová et al., 2007b; Yousuf et al., 2015). Some studies pointed out that the e3 allele is the most frequent in all human groups, while \textit{APOE} e3/e3 is the most common genotype in most population (Corbo and Scacchi, 1999; Al-Dabbagh et al., 2009; Achourirassas et al., 2016; Jairani et al., 2016; Monge-Argilés et al., 2016; Tanyanyiwa et al., 2016).

Meizhou is a city covering the northeast of Guangdong Province, which connects to Fujian, Guangdong, and Jiangxi provinces, with an area of 15,876 km\textsuperscript{2} and a popula-
tion of 5.44 million. The vast majority of the residents living in this area are Hakka. Hakka is an intriguing Han Chinese population that mainly inhabits southern China and that migrated south originally from the Reaches of Yellow River (Li, 1997). There is currently no data about the genetic variations of APOE gene in the Hakka population.

Materials and Methods

Subjects

For this study, 6,907 Chinese Hakka subjects were included through February 2016 to August 2017. Subjects visited Meizhou People’s Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University located in Guangdong province in China. The present study was performed in accordance with the ethical standards laid down in the updated version of the 1964 Declaration of Helsinki and approved by Human Ethics Committees of Meizhou People’s Hospital. All the patients had signed the informed consent.

DNA extraction

Blood samples were stored in 2-mL vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) from each participant. Genomic DNA was extracted from the samples using QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer’s instructions. DNA concentration and purity were quantified using NanoDrop 2000™ Spectrophotometer (ThermoFisher Scientific, Waltham, MA), and only good quality DNA (A260/280 ratio > 1.7) was stored at -80 °C up to the day of analysis.

Polymerase chain reaction and genotyping

The single nucleotide polymorphisms of APOE gene rs429358 and rs7412 were genotyped using a commercially available kit (Sinochips Bioscience Co., Ltd, Zhuhai, Guangdong, China). PCR assays was performed according to the following protocol: 50 °C for 2 min, pre-denaturation at 95 °C for 15 min, followed by 45 cycles at 94 °C for 30 s and 65 °C for 45 s. The amplified products were revealed using an APOE Gene typing Detection kit (gene chip assay) (Sinochips Bioscience Co., Ltd, Zhuhai, China).

Statistical analysis

Frequencies of the ε2, ε3 and ε4 alleles were calculated by gene counting, e.g., the frequency of ε2=(2* APOE ε2/ε2 + APOE ε2/ε3 + APOE ε2/ε4)/ total number of alleles.

SPSS statistical software version 19.0 was used for data analysis. The data are reported as the means ± SD. Chi-square and Fisher’s exact tests were used to compare the allele and genotype frequencies. Descriptive analysis was used to compare allele frequencies between the Hakka population and published data of other ethnic groups. A value of p < 0.05 was considered as statistically significant.

Results

A total of 6,907 subjects, 4,366 (63.21%) men and 2,541 (36.79%) women, were recruited in the study. The sample age ranged from 1 to 101 (64.06 ± 14.68) years, with means of 63.48 ± 14.62 in men and 65.06 ± 14.74 in women. Most of them came from southern China including seven areas of Meizhou city, Guangdong Province and some regions of Jiangxi Province, all of them are Hakka. The geographical position of Meizhou city is shown in Figure 1.

In this study, the genotype ε3/ε3 accounted for 65.43% followed by ε2/ε3 (15.85%), ε3/ε4 (14.13%), ε2/ε4 (3.01%), ε4/ε4 (0.84%), and ε2/ε2 (0.74%) in all subjects; ε3 had the greatest allele frequency (80.42%) followed by ε2 (10.17%) and ε4 (9.41%). The results as showed in Table 1.

Figure 1 - Geographical position of Meizhou in Guangdong Province of China.
Allele frequency (80.42%) followed by \( \varepsilon_3 \) and \( \varepsilon_4 \) and \( \text{APOE} \) for other populations. (9.41%). This was consistent with previous research on Comparison of our results with the geographically neighboring countries showed that the frequencies of \( \varepsilon_4 \) allele in Chinese populations were lower than in Javanese (Svobodova et al., 2007a,b) populations, while the frequencies of the \( \varepsilon_2 \) allele were higher and of the \( \varepsilon_4 \) allele lower than in Japanese (Hallman et al., 1991; Gerdes et al., 1992) and Koreans (Hong et al., 1997). In addition, the analysis showed that the frequencies of \( \varepsilon_2 \) and \( \varepsilon_4 \) allele in Hakka population were similar to the Vietnamese (Nghiem et al., 2004), Chinese-Shanghai (Yang et al., 2003), Chinese-Kunming Han (Tang et al., 2005), Chinese-Northeast (Zhou et al., 2005), and French (Boerwinkle et al., 1986; Gueguen et al., 1989; Bailleul et al., 1993).

Comparing our results with other Chinese populations, the frequencies of the \( \varepsilon_2 \) and \( \varepsilon_4 \) alleles in the Hakka population were highly similar to the Chinese-Shanghai, Chinese-Kunming Han, and Chinese-Northeast, while the frequency of \( \varepsilon_2 \) in the Hakka population was higher than Chinese-Dehong Dai (Tang et al., 2005) and Chinese-Jiangsu Han (Liang et al., 2009) (Figure 2). This suggests that the risk of some diseases in the Hakka population of Southern China may be different from those of other populations. Since \( \varepsilon_4 \) polymorphism is associated with increased risk of cardiovascular disease, Alzheimer’s disease, and other diseases, our findings suggest a low genetic risk in the Hakka population for these diseases.

In some reports, the subjects were relatively few and the results did not represent the actual gene frequencies of that region and population. Here, the Apolipoprotein E genetic polymorphism was analyzed in a large ethnic Hakka population in southern China, and is the first performed on a large sample of the population of this area. Our sample size is one of the largest of all studies, and thus should more accurately assess the \( \text{APOE} \) gene allele and genotype frequencies of the Hakka population in southern China. Our next step is to increase the sample size of the study. A number of investigations have demonstrated that carriers of \( \varepsilon_4 \) allele are characterized by a lower life expectancy (Hyman et al., 1996; Gerdes et al., 2015). Thus, we are going to investigate the \( \text{APOE} \) gene polymorphisms in people living in Jiaoqing, which is considered the hometown of longevity in China.

### Discussion

ApoE is one of the important apolipoproteins in plasma, which is mainly synthesized, secreted, and metabolized in the liver (Schneider et al., 1981; Rall Jr et al., 1982b). It is involved in the transport, storage, and metabolism of lipids, and has the effects of repairing tissues, inhibiting platelet aggregation, and regulating immunity (van den Elzen et al., 2005). Studies have found that \( \text{APOE} \) gene polymorphisms are closely associated with coronary heart disease, hyperlipidemia, cerebral infarction, Alzheimer’s disease, multiple sclerosis, chronic hepatitis, and other diseases (Ghiselli et al., 1981; Corder et al., 1993; Faivre et al., 2005; Price et al., 2006; Rovin et al., 2007; Kathiresan et al., 2008). ApoE4 is associated with decreased longevity, increased plasma total and LDL cholesterol, and increased prevalence of cardiovascular disease and Alzheimer’s disease. Different populations have different frequencies of genetic polymorphisms of \( \text{APOE} \) (Gerdes et al., 1996).

In most populations, \( \varepsilon_3/\varepsilon_3 \) is the commonest genotype while \( \varepsilon_3 \) is the commonest allele. In this study, genotype \( \varepsilon_3/\varepsilon_3 \) accounted for 65.43% followed by \( \varepsilon_2/\varepsilon_3 \) (15.85%), \( \varepsilon_3/\varepsilon_4 \) (14.13%), \( \varepsilon_2/\varepsilon_4 \) (3.01%), \( \varepsilon_4/\varepsilon_4 \) (0.84%), and \( \varepsilon_2/\varepsilon_2 \) (0.74%) in all subjects. \( \varepsilon_3 \) allele had the greatest allele frequency (80.42%) followed by \( \varepsilon_2 \) (10.17%) and \( \varepsilon_4 \) (9.41%). This was consistent with previous research on other populations.

We compared the allele frequencies estimated here for \( \text{APOE} \) \( \varepsilon_2 \), \( \varepsilon_3 \), and \( \varepsilon_4 \) allele with respect to previously published reports in other ethnic populations (Table 2). Comparison of our results with the geographically neighboring countries showed that the frequencies of \( \varepsilon_4 \) allele in Chinese populations were lower than in Javanese (Svobodova et al., 2007a,b) populations, while the frequencies of the \( \varepsilon_2 \) allele were higher and of the \( \varepsilon_4 \) allele lower than in Japanese (Hallman et al., 1991; Gerdes et al., 1992) and

### Table 1 - Allele and genotype frequencies of \( \text{APOE} \) in 6907 participants in Hakka population.

| Allele | Male (n=4366) | Female (n=2541) | Combined (n=6907) |
|--------|---------------|----------------|------------------|
| n      | Frequency %   | n              | Frequency %      | n              | Frequency %  |
| \( \varepsilon_2 \) | 899           | 0.103           | 506              | 0.100           | 1405         | 0.102         |
| \( \varepsilon_3 \) | 7016          | 0.803           | 4093             | 0.805           | 11109        | 0.804         |
| \( \varepsilon_4 \) | 817           | 0.094           | 483              | 0.095           | 1300         | 0.094         |

| Genotype | Male (n=4366) | Female (n=2541) | Combined (n=6907) |
|----------|---------------|----------------|------------------|
| n        | Frequency %   | n              | Frequency %      | n              | Frequency %  |
| \( \varepsilon_2/\varepsilon_2 \) | 29            | 0.66           | 22               | 0.87           | 51           | 0.74         |
| \( \varepsilon_2/\varepsilon_3 \) | 710           | 16.26          | 385              | 15.15          | 1095         | 15.85        |
| \( \varepsilon_2/\varepsilon_4 \) | 131           | 3.00           | 77               | 3.03           | 208          | 3.01         |
| \( \varepsilon_3/\varepsilon_3 \) | 2851          | 65.30          | 1668             | 65.64          | 4519         | 65.43        |
| \( \varepsilon_3/\varepsilon_4 \) | 604           | 13.83          | 372              | 14.64          | 976          | 14.13        |
| \( \varepsilon_4/\varepsilon_4 \) | 41            | 0.94           | 17               | 0.67           | 58           | 0.84         |
Table 2 - Distribution of APOE (ε2, ε3, ε4) allele frequencies among major study populations.

| Populations         | Total Number | Alleles frequency of APOE | References                          |
|---------------------|--------------|---------------------------|------------------------------------|
|                     |              | ε2    | ε3    | ε4    |                          |
| **Asians**          |              |       |       |       |                          |
| Chinese             |              |       |       |       |                          |
| Chinese-Hakka       | 6907         | 0.102 | 0.804 | 0.094 | This work                |
| Chinese-Shanghai    | 266          | 0.098 | 0.786 | 0.116 | Yang et al., 2003        |
| Chinese-Dehong Dai  | 171          | 0.064 | 0.889 | 0.047 | Tang et al., 2005        |
| Chinese-Jinangsu Han| 168          | 0.071 | 0.863 | 0.066 | Liang et al., 2009       |
| Chinese-Kunming Han | 71           | 0.092 | 0.852 | 0.056 | Tang et al., 2005        |
| Chinese-Northeast   | 69           | 0.096 | 0.824 | 0.081 | Zhou et al., 2005        |
| Indian              | 4450         | 0.039 | 0.887 | 0.073 | Thelma et al., 2001      |
| Japanese            | 1097         | 0.048 | 0.851 | 0.101 | Hallmann et al., 1991; Gerdes et al., 1992 |
| Mongolian           | 744          | 0.037 | 0.808 | 0.155 | Svobodová et al., 2007a  |
| Vietnamese          | 348          | 0.090 | 0.790 | 0.120 | Ngiem et al., 2004       |
| Malay               | 223          | 0.140 | 0.620 | 0.240 | Gajra et al., 1994a      |
| Javanese            | 197          | 0.060 | 0.770 | 0.170 | Gajra et al., 1994b      |
| Koreans             | 145          | 0.020 | 0.870 | 0.110 | Hong et al., 1997        |
| Iranian             | 129          | 0.027 | 0.912 | 0.061 | Raygani et al., 2005     |
| **Europeans**       |              |       |       |       |                          |
| Dutch               | 2318         | 0.085 | 0.752 | 0.163 | Smit et al., 1988; Knijff et al., 1993 |
| Finnish             | 2245         | 0.044 | 0.748 | 0.208 | Lehtimäki et al., 1990; Salo et al., 1993; Hallman et al., 1991 |
| Germans             | 1211         | 0.083 | 0.784 | 0.133 | Kolovou et al., 2009     |
| Italians            | 2000         | 0.060 | 0.849 | 0.091 | Corbo et al., 1995       |
| Spanish             | 1286         | 0.052 | 0.856 | 0.091 | Valveny et al., 2010; Gerdes et al., 1992; Lucotte et al., 1997; Muros and Rodríguez-Ferrer, 1996 |
| French              | 1228         | 0.108 | 0.771 | 0.121 | Bailleul et al., 1993; Gueguen et al., 1989; Boerwinkle et al., 1986 |
| Belgians            | 189          | 0.069 | 0.762 | 0.160 | Engelborghs et al., 2003 |
| UK                  | 734          | 0.089 | 0.767 | 0.144 | Corbo et al., 1995; Lucotte et al., 1997 |
| Greeks              | 551          | 0.054 | 0.878 | 0.068 | Marios et al., 1995; Sklavounou et al., 2010 |
| Danish              | 466          | 0.085 | 0.741 | 0.174 | Gerdes et al., 1992     |
| Swedish             | 407          | 0.077 | 0.740 | 0.190 | Roussos et al., 2004     |
| Turks               | 90           | 0.063 | 0.868 | 0.069 | Brega et al., 1998      |
| **Africans**        |              |       |       |       |                          |
| Nigeria             | 1562         | 0.064 | 0.684 | 0.252 | Kamboh et al., 2015      |
| Algerian            | 732          | 0.050 | 0.846 | 0.104 | Boulenouar et al., 2013  |
| Sub-Saharans        | 470          | 0.116 | 0.706 | 0.178 | Zekraoui et al., 1997    |
| Nigerians           | 365          | 0.027 | 0.677 | 0.296 | Sepehnia et al., 1989    |
| Khoi San            | 247          | 0.077 | 0.553 | 0.370 | Sandholzer et al., 1995  |
| **North Americans** |              |       |       |       |                          |
| American- whites    | 702          | 0.082 | 0.778 | 0.140 | Djoussé et al., 2004     |
| **South Americans** |              |       |       |       |                          |
| Brazil              | 2010         | 0.063 | 0.797 | 0.140 | Fuzikawa et al., 2007; França et al., 2004; Brito et al., 2011; Souza et al., 2003 |
| Venezuela           | 1841         | 0.055 | 0.834 | 0.111 | Molero et al., 2001; Arráiz et al., 2010 |
| Colombia            | 1001         | 0.075 | 0.814 | 0.111 | Velez-Pardo et al., 2015 |
Conclusions

The frequencies of the ε4 allele in Chinese populations were lower than in Mongolians and Javanese, while the frequencies of the ε2 allele were higher and of the ε4 allele lower than in Japanese and Koreans, which are geographically neighboring countries. The frequencies of the ε2 and ε4 alleles in the Hakka population were similar to the Vietnamese, Chinese-Shanghai, Chinese-Kunming Han and Chinese-Northeast, and French, while the frequency of ε2 in the Hakka population was higher than Chinese-Dehong Dai and Chinese-Jiangsu Han. Our findings suggest a low genetic risk in the Hakka population for some diseases.

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