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CETP polymorphisms confer genetic contribution to centenarians of Hainan, south of China

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ABSTRACT

Objective: In this paper, we will discuss if the CETP polymorphism contributes to the centenarians in Hainan island.**Methods:** We tested the *TaqIB* and *I405V* polymorphisms of CETP gene among 276 centenarians and 301 matched healthy individuals by AS-PCR and analyzed the data with SPSS software package (Version 19.0).**Results:** Our data indicated that allele B1 and V have the significant differences between centenarians and healthy control groups with $P < 0.001$. Further analysis implied that genotypes B1B1 ($P < 0.001$, $OR = 0.148$, $95\% CI = 0.095–0.230$) and VV ($P < 0.001$ and $OR = 0.353$, $95\% CI = 0.237–0.525$) were significantly different between centenarians and matched controls. The combination of B and V, such as B1B1-II ($P < 0.001$, $OR = 0.128$, $95\% CI = 0.049–0.329$), B1B1-IV ($P < 0.001$, $OR = 0.115$, $95\% CI = 0.056–0.237$), B1B2-VV ($P < 0.05$, $OR = 0.534$, $95\% CI = 0.310–0.920$), and B2B2-VV ($P < 0.001$, $OR = 0.198$, $95\% CI = 0.086–0.453$) have significant differences between centenarians and matched healthy individuals from Hainan.**Conclusion:** Our results implied that allele B1B1 and VV, as well as the combination B1B1-II, B1B1-IV, B1B2-VV and B2B2-VV may contribute to the longevity in centenarians of Hainan, south of China.

1. Introduction

Aging is a complicated physical process with the function reducing for many organisms in line with the increasing life span, such as the decreasing cellular proliferative potential, receding efficient immune system, and changing of endocrine system functions [1,2]. Centenarians have surpass 20–25 years life span than general older population, which indicated that the centenarians have the unique capability for postponing or even evading the onset of disease life, thus the centenarians could take as the model of healthy aging and investigate the aging related diseases [3]. Genetic factors contributed around 25% heritability of the life span [4,5], such as the CETP gene [6], relative higher of mitochondrial DNA content [7]. Thus, genetic factors were proposed as essential factors on affecting the life span of human beings [8,9].

CETP is a hydrophobic glycoprotein, it transfers the excess cholesterol esters (CE) of vessel wall to liver by the reverse cholesterol transport (RCT) pathway [6]. It modifies high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoprotein (VLDL) levels by transferring CE from CE rich particles including HDL and LDL to triglyceride rich particles, such as VLDL, for exchanging of triglyceride with the latter [10,11]. Previous works have revealed that the mutation of CETP gene can change its activity by affecting the retrograde transport of HDL, which is also increase the incidence of coronary atherosclerotic heart disease (CHD) [12]. Interestingly, previous works have revealed the higher high-density lipoprotein levels in longevities [13], and the genetic polymorphisms of CETP gene were also deemed as one of the protection factors for longevity by changing the structure of the proteins and causing the changes for the function of CETP gene [14,15], although there were still some debates on whether the polymorphisms of CETP gene could protect the longevity in centenarians from Italian [16], Chinese [14] and Japanese [17]. The small sample size may cause the inconsistent results [18]. Significantly, in our previous work, we found the significantly lower triglyceride and total cholesterol in 535 centenarians of

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Hainan, south of China by comparing with those of the general older group, which implied that the polymorphisms of CETP gene may relate with the longevity of Hainan, south of China. Our previous works have revealed the significantly different genetic polymorphism of CETP gene among centenarians and health control [19,20]. However, there was still lacking of the research work to investigate whether the combination of TaqIB and I405V polymorphisms of CETP gene contribute the centenarians of Hainan, the home of centenarians from south of China.

Hainan locates in the South China Sea, separated from Leizhou Peninsula of Guangdong's to the north by the shallow and narrow Qiongzhou Strait. Hainan, the home of longevity, has around 619 centenarians and the centenarian rate in Hainan was around 8/100000 by 2009 [19]. Therefore, the aim of this study was to determine the TaqIB and I405V polymorphisms of CETP gene and its relationship with centenarians from Hainan, south of China. The TaqIB and I405V polymorphisms of CETP gene were detected among 276 centenarians and 301 matched healthy individuals with AS-PCR. Further statistics analysis of the allele frequency and combination for genotype of the two variations of CETP gene were performed between the centenarians and matched healthy control groups in this study.

2. Material and methods

2.1. Sampling

Totally, blood samples of 276 centenarians and 301 healthy control subjects were recruited from Hainan province, and the healthy controls were recruited from the same region with the centenarians, the ancestors for the healthy control individuals all have lived with the centenarians more than 100 years. The ages for the centenarians and the matched health control were recorded from the department of civil affairs in Hainan province, and the mean age for the centenarians was 102.0 ranging from 100 to 105 years old, and the ages of healthy control was 62.8 with a range between 58 and 70 years old. The sex ration for the centenarians was 246/33 for female and male, which was 136/165 in matched healthy control group. Informed consents were performed according to the tenets of the Declaration of Helsinki and following the guidance of sample collection of Human Genetic Disease (863 program) by the Ministry of Public Health of China. The written informed consents were signed by the

participants or their guardian before collecting of the blood samples. This work was approved by the Ethics Committee of Hainan Medical College.

2.2. DNA isolation, PCR amplification and genotyping

The genomic DNA was isolated from whole blood with standard phenol/chloroform method. The PCR fragments contain the polymorphisms TaqIB and I405V, which were amplified with the primer pairs as described in our previous work [21]. Two independent PCR for each variation of the same individual were amplified with the prime pair containing the specific mutation and the wild type sequence, the latter was used to confirm the authenticity of the variation detected with the primers containing the specific mutation. The PCR reaction were performed in the 25 μ L of the reaction mixture including 2.6 μ L PCR Buffer II (Mg^{2+} Plus), 400 μ M of each dNTP, 0.2 μ M for each primer, 2.5 units of TaKaRa Taq (TaKaRa Bio Inc., Dalian, China), as well as 50 ng DNA. The amplification was performed on the ABI GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) by denaturing cycle of 94 $^{\circ}$ C for 1 min; 30 amplification cycles for 94 $^{\circ}$ C for 30 s, 63 $^{\circ}$ C for 50 s, 72 $^{\circ}$ C for 1 min; and one full extension cycle of 72 $^{\circ}$ C for 10 min. The PCR products were analyzed by 2% gel electrophoresis with ethidium bromide.

2.3. Data analysis

Genotype and allele frequencies were recorded with Excel of Microsoft office software package. Pearson's chi-square test with a one degree of freedom was used to assess the significant differences between centenarians and matched healthy control groups by taking 0.05/0.001 as the cut off value with SPSS software package (Version 19.0).

3. Results

3.1. Identification of the polymorphisms of CETP genes with AS-PCR

In this work, both the variation of TaqIB and I405V were detected among 276 centenarians and 301 matched health individuals with AS-PCR. According to the principle of AS-PCR,

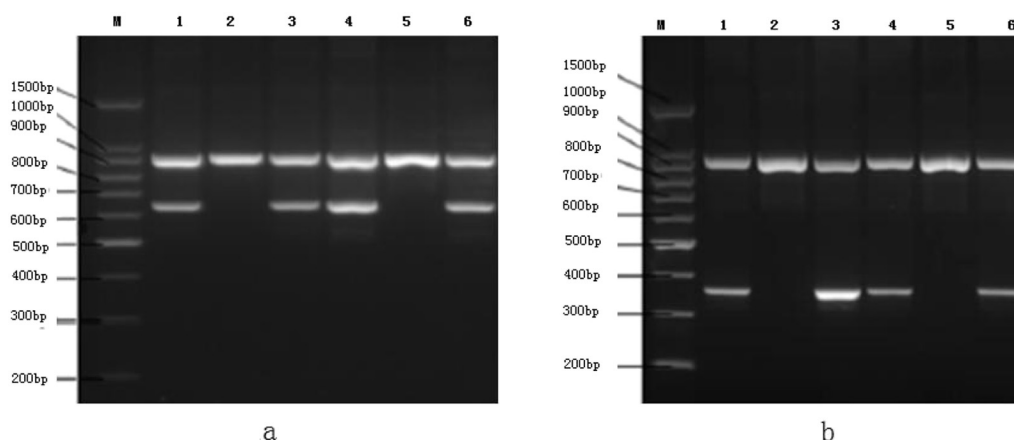


Figure 1. The gel electrophoresis for polymorphisms TaqIB and I405V. TaqIB and I405V were listed in a and b, respectively. The marker were ranged from 200 bps to 1500 bps, which was, 200 bps, 300 bps, 400 bps, 500 bps, 600 bps, 700 bps, 800 bps, 900 bps, 1000 bps and 1500 bps, respectively.

Table 1

The allele frequency and genotype frequency of polymorphism TaqIB in CETP gene.

Genotype	Centenarians (%)	Control (%)	P	χ^2	OR	95% CI
B1B1	30 (10.87)	136 (45.18)	0.000	82.722	0.148	0.095–0.230
B1B2	202 (73.19)	120 (39.87)	0.000	55.4	3.753	2.631–5.354
B2B2	44 (15.94)	45 (14.95)	0.742	0.109	1.079	0.687–1.695
B1	262 (47.46)	392 (65.12)	0.000	36.543	0.484	0.382–0.613
B2	290 (52.54)	210 (34.88)	0.724	0.125	1.043	0.827–1.314

to identify the polymorphism of each position, two independent PCR actions were performed for each individual with the primer pair including variation type and wild type sequence, respectively. The PCR products were analyzed by 2% gel electrophoresis with ethidium bromide, the different types of the TaqIB and I405V were listed in Figure 1 to identify the different type of the two mutations. As shown in Figure 1a, the variation TaqIB were detected, for the lane 1 and 2 showed the genotype of B1B1, lanes 3 and 4 indicate the type of B1B2 and the lanes 5 and 6 illustrated the type B2B2. The polymorphisms of I405V were demonstrated in Figure 1b, in detail, lanes 1 and 2 showed genotype II, lanes 3 and 4 demonstrated genotype IV, and lanes 5 and indicated the genotype VV.

3.2. Allele and genotypes frequency for TaqIB and I405V of CETP gene in centenarians and matched health controls

For the TaqIB polymorphism, as shown in Table 1, the allele B1 has a lower frequency (262/552, 47.46%) than that of allele B2 (290/552, 52.54%) in centenarians group, this was in contrast with that of match health control group; in the later, allele B1 (392/602, 65.12%) was higher than allele B2 (210/602, 34.88%) (Table 1). The B1B2 (202/276, 73.19%) was the predominant genotype of centenarians, and B1B1 (136/301, 45.18%) following. B2B2 has the lower frequency in both centenarians (44/276, 15.94%) and health control (45/301, 14.95%) groups (Table 1). As shown in Table 2, for the I405V polymorphism,

our results showed that there was different pattern with that of TaqIB, I was predominant allele (310/552, 56.16%) than V (342/552, 43.84%) in centenarians group; allele V was predominant (360/602, 59.80%) than allele I (242/602, 40.20%) in healthy control group. Interestingly, the genotype IV was the major component both in centenarians (152/276, 55.07%) and healthy control (146/301, 48.50%) groups. In contrast, the genotype II was higher in centenarians group than that of health group, genotype VV has the reverse pattern with genotype II in centenarians and healthy control group. Further, the combination for polymorphisms TaqIB and I405V were calculated, our data showed that B1B2-VV has the highest frequency in centenarians group (123/276, 44.57%) and healthy control group (68/301, 22.59%), in addition, the combination of B1B1 and IV also has the similar frequency in healthy control group with B1B2-IV. The B2B2-II was only detected among the centenarians group (17/276, 6.16%).

3.3. Association for TaqIB and I405V of CETP gene between centenarians and matched health controls

To further evaluate the correlations between the two polymorphisms TaqIB and I405V of CETP gene and longevity in centenarians from Hainan, south of China, the statistic analysis was performed, our data showed that allele B1 and V have the significant differences between centenarians and healthy control groups ($P < 0.001$, $OR = 0.484$, $95\% CI = 0.382-0.613$; $P < 0.001$, $OR = 0.525$, $95\% CI = 0.415-0.663$, respectively),

Table 2

The allele frequency and genotype frequency of polymorphism I405V in CETP gene.

Genotype	Centenarians (%)	Control (%)	P	χ^2	OR	95% CI
II	79 (28.62)	48 (15.95)	0.000	13.478	2.114	1.411–3.166
IV	152 (55.07)	146 (48.50)	0.115	2.487	1.301	0.938–1.806
VV	45 (16.30)	107 (35.55)	0.000	27.479	0.353	0.237–0.525
I	310 (56.16)	242 (40.20)	0.000	29.395	1.906	1.508–2.408
V	242 (43.84)	360 (59.80)	0.000	29.395	0.525	0.415–0.663

Table 3

The combination for genotypes of TaqIB and I405V frequency in CETP gene.

Haplotype	Centenarians (%)	Control (%)	P	χ^2	OR	95% CI
B1B1-II	5 (1.81)	38 (12.62)	0.000	24.408	0.128	0.049–0.329
B1B1-IV	9 (3.26)	68 (22.59)	0.000	46.524	0.115	0.056–0.237
B1B1-VV	16 (5.80)	30 (9.97)	0.065	3.412	0.556	0.296–1.044
B1B2-II	57 (20.65)	10 (3.32)	0.000	42.131	7.574	3.782–15.168
B1B2-IV	123 (44.57)	68 (22.59)	0.000	31.394	2.755	1.923–3.947
B1B2-VV	22 (7.97)	42 (13.95)	0.022	5.225	0.534	0.310–0.920
B2B2-II	17 (6.16)	0	0.000	19.103	–	–
B2B2-IV	20 (7.25)	10 (3.32)	0.034	4.498	2.273	1.045–4.947
B2B2-VV	7 (2.54)	35 (11.63)	0.000	17.633	0.198	0.086–0.453

the allele I also has significant differences between centenarians and healthy control group at $P < 0.001$; for the six genotypes of TaqIB and I405V, the data indicated that genotypes B1B1 ($P < 0.001$, $OR = 0.148$, $95\% CI = 0.095–0.230$), B1B2 ($P < 0.001$, $OR = 3.753$, $95\% CI = 2.631–5.354$), II ($P < 0.001$, $OR = 2.114$, $95\% CI = 1.411–3.166$) and VV ($P < 0.001$, $OR = 0.353$, $95\% CI = 0.237–0.525$). As the combination of TaqIB and I405V, the data showed that, except the combination of B1B1-VV, 8 of 9 combination for the two polymorphisms have differed between centenarians and control groups ($P < 0.05$) (Table 3); furthermore, 6 of 9 which has significant differences between the two populations, including B1B1-II, B1B1-IV, B1B2-II, B1B2-IV, B2B2-II and B2B2-VV ($P < 0.001$).

4. Discussion

CETP plays a key role in lipoprotein metabolism and modulation of HDL-C, it is associated with HDL-C levels more strongly than the other loci across the genome [10,12]. Previous works have revealed the correlation between the gene polymorphisms of CETP and carotid atherosclerosis [22,23] and longevity [6,24,25]. However, there were still some debates on whether the polymorphism of genotype B2B2 of TaqIB and VV of I405V for CETP gene performed protecting roles for longevity [14,15,18,26]. The small sample size may cause the controversial results [18], as well as the fact that different longevity group may have different genetic backgrounds, thus, in this study, to resolve the aforementioned problems, we screened the polymorphisms of TaqIB and I405V among 276 centenarians and 301 matched healthy control groups.

Our data indicated that allele B1 and V has the significant difference between centenarians and healthy control groups with $P < 0.001$. Further analysis for the genotypes of the two polymorphisms showed that B1B1 ($P < 0.001$) and VV ($P < 0.001$) both have the significant differences between the centenarians and health control groups, taken together with the OR values for B1B1 ($OR = 0.148$, $95\% CI = 0.095–0.230$) and VV ($OR = 0.353$, $95\% CI = 0.237–0.525$) less than 1, which implied that these two genotypes may contribute the genetic factors of longevity in centenarians from Hainan, south of China. However, there was no significant difference for B2B2 between Hainan centenarians and matched control group, this was in line with that of Bama centenarians [14], which resident in Guangxi, south of China, which indicated the similar genetic backgrounds for the centenarians from the similar geographic regions. The results for polymorphism I405V was in line with previous works, which supported the protection role of genotype VV, our data was in line with that from previous work [19,20]. However, this was different from the longevity from Italians [16], and the meta analysis results for Chinese longevity [6], which indicated that the longevity from China may have different genetic backgrounds.

In consideration of the complication of the longevity, thus, we proposed that the combination of the two polymorphisms of CETP gene may contribute the longevity of centenarians from Hainan, south of China. Thus, in this study, 9 of the combination of genotype TaqIB and I405V were calculated and analyzed. With the exception of B1B1-VV, our data indicated that 8 of 9 combination for the two polymorphisms have significantly differences between centenarians and control groups ($P < 0.05$), which implied that the combination of two genotype may contribute the longevity from

Hainan, in detail, the combination of B1B1-II ($P < 0.001$, $OR = 0.128$, $95\% CI = 0.049–0.329$), B1B1-IV ($P < 0.001$, $OR = 0.115$, $95\% CI = 0.056–0.237$), B1B2-VV ($P < 0.05$, $OR = 0.534$, $95\% CI = 0.310–0.920$), B2B2-VV ($P < 0.001$, $OR = 0.198$, $95\% CI = 0.086–0.453$) all have the OR value less than 1, which implied that the combination for the two polymorphisms of CETP gene may contribute the protection of longevity of centenarians from Hainan, south of China.

In summary, in this study, to illustrate the genetic patterns of polymorphisms of TaqIB and I405V of CETP gene in Hainan centenarians and evaluate the correlation between polymorphisms of TaqIB and I405V of CETP gene and centenarians from Hainan, the aforementioned two polymorphisms were screened among 276 centenarians and 301 healthy control individuals from Hainan, south of China. Our results indicated that alleles B1 and V in centenarians were significant different from that of healthy control individuals, further statistical analysis indicated that genotype B1B1 and VV may contribute the protection role on the longevity of centenarians from Hainan, south of China. Furthermore, the statistical analysis for the combination of different genotypes of TaqIB and I405V showed that B1B1-II, B1B1-IV, B1B2-VV and B2B2-VV were the important genetic factor affecting the longevity of Hainan, they may confer the protection role of longevity for centenarians from Hainan, south of China.

Conflict of interest statement

No conflict was declared for all authors.

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