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ORIGINAL ARTICLE

Effect of Apolipoprotein E Genotypes on Huntington's Disease Phenotypes in a Han Chinese Population

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Abstract Huntington's disease (HD) is an autosomal dominant degenerative disease that mainly encompasses movement, cognition, and behavioral symptoms. The apolipoprotein E (*APOE*) gene is thought to be associated with many neurodegenerative diseases. Here, we enrolled a cohort of 223 unrelated Han Chinese patients with HD and 1241 unrelated healthy controls in Southeastern China and analyzed the correlation between *APOE* genotypes and HD phenotypes. The results showed that the frequency of the E4 allele (7.1%) in HD patients was statistically less than that in controls (12.0%) (P =0.004). In addition, we divided patients into motor-onset and non-motor-onset groups, and analyzed the relationship with *APOE* genotypes. The results, however, were negative. Furthermore, the age at onset (AAO), defined as the age at the onset of motor symptoms, was compared in each *APOE* genotype subgroup and multivariate regression analysis was used to exclude the interference of CAG repeat length on AAO, but no association was found between *APOE* genotypes and AAO. Finally, we analyzed adult-onset HD to exclude the interference caused by juvenile HD (n = 13), and the results were negative. Therefore, our study suggests that *APOE* may not be a genetic modifier for HD, especially for adult-onset HD among Chinese of Han ethnicity. To the best of our knowledge, this is the first study of the correlation between *APOE* genotypes and HD phenotypes in a Han Chinese population.

Keywords Huntington's disease · Phenotype · Apolipoprotein E · Han Chinese population

Xiao-Yan Li, Yan-Bin Zhang and Miao Xu have contributed equally to this work.

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Introduction

Huntington's disease (HD) is an age-related, autosomal dominant neurodegenerative disease resulting from the expansion of a CAG repeat in the first exon of the huntingtin gene (*HTT*). It is typically characterized by motor dysfunction, cognitive impairment, and psychiatric disorders [1]. Atypical clinical features also occur, such as ataxia, tics, tremor, dystonia, and parkinsonism [2]. In the general East-Asian population, the average CAG repeat size is 16.9 repeats, which is lower than the 17.8 repeats in Europeans due to the specific haplogroup [3]. Individuals who inherit 36–39 CAG repeats may have reduced and mild penetrance, and those who inherit 40 CAG repeats or more have complete penetrance [4]. It has been

demonstrated that CAG repeat length is inversely associated with the age at onset (AAO) [5, 6]. Although CAG repeat length constitutes the most important factor affecting AAO, this only accounts for 50%–72% of its variance [7, 8]. Furthermore, individuals with the same CAG repeats can present variable AAOs, symptoms, and disease durations. Many factors could affect the phenotypes of the disease, such as genetic modifiers and environment factors. In this study, our main purpose was to study the correlation between apolipoprotein E *(APOE*) genotypes and HD phenotypes.

ApoE protein, coded by the *APOE* gene, is a serum glycoprotein consisting of 299 amino-acid residues. It is secreted as a protein with a relative molecular mass of 34,200 [9]. ApoE regulates lipid metabolism by mediating lipid transport from one place to another [10]. In the central nervous system, ApoE is primarily produced by astrocytes, and mediates cholesterol delivery between neurons. This is an essential process for axonal growth, synapse formation, and remodeling, which are crucial for cognitive functions and neuronal repair [11, 12].

The *APOE* gene has three common haplotypes – E2, E3, and E4 – which encode three corresponding protein isoforms. These haplotypes give rise to six genotypes, the most common of which is E3E3 [13]. The three protein isoforms are distinguished from each other by the interchange of Cys and Arg residues at positions 112 and 158 of the protein. E2 has Cys residues in both positions, E3 has Cys-112 and Arg-158, and E4 has Arg in both positions. The single amino-acid differences at the two positions influence the structure of the ApoE isoforms, thus altering their affinity for lipids, receptors, and beta-amyloid (Aβ) [14]. Aβ is the main component of plaques, which is one of the typical pathological changes in Alzheimer's disease (AD) [15]. The other pathological change is the neurofibrillary tangles composed of hyperphosphorylated tau [16]. Several lines of evidence show that ApoE isoforms play differential roles in Aβ aggregation and clearance [14, 17]. In addition, it has been reported that ApoE is also involved in tau pathogenesis and tau-mediated neurodegeneration [18].

Many studies have shown that the *APOE* E4 allele is a risk factor for developing AD, Pick's disease, and dementia with Lewy bodies (DLB) [19–21]. Moreover, recent research has demonstrated that the E4 allele can independently trigger inflammatory cascades, which can break down the blood-brain barrier (BBB) and admit blood-derived toxic proteins into the brain [22]. BBB breakdown leads to neuronal impairment, synaptic dysfunction, and neurodegeneration. Based on these findings, the E4 allele is considered to be one of the pathogenic mechanisms of AD, Pick's disease, DLB, and other degenerative disorders.

All these studies [14, 17, 22] indicate that *APOE* might be a candidate gene in modifying the phenotypes of neurodegenerative diseases. Considering that HD is a degenerative disorder and has various clinical symptoms, we aimed to investigate the correlation between *APOE* genotypes and HD phenotypes. In previous studies, most researchers focused on the influence of *APOE* on AAO in HD patients of Caucasian origin [23, 24]. To date, there is no large-scale study of Chinese HD patients of Han ethnicity.

Materials and Methods

Participants

Between January 2008 and August 2017, 223 unrelated patients of Han Chinese origin with HD confirmed by genetic testing of the *HTT* gene were recruited. They were enrolled at the Departments of Neurology in three medical centers, The Second Affiliated Hospital of Zhejiang University School of Medicine (Hangzhou), Huashan Hospital Affiliated to Fudan University (Shanghai), and The First Affiliated Hospital of Fujian Medical University (Fuzhou). Most patients came from Southeastern China. This study was approved by the Ethics Committees of the above hospitals. Patients or their guardians gave informed consent. Also, 1241 unrelated aged individuals (≥65 years old) from communities without any history of neurodegenerative disease (such as HD, AD, or Parkinson's disease) were included as the control group. All the control individuals denied positive family histories or cognitive impairment.

Genetic Analysis of CAG Repeats in the *HTT* Gene

Genomic DNA was extracted from peripheral blood using a DNA extraction kit (Qiagen Inc., Valencia, CA). Genetic testing for the *HTT* gene was conducted for all affected individuals using the method described previously [25]. The forward primer was 5′-CAGAGCCCCATTCATTGCC-3′ and reverse was 5′-TGAGGAAGCTGAGGAGGC-3′. CAG-tract repeat sizes were determined by Sanger sequencing using a procedure described previously [26].

Genotyping of *APOE*

APOE genotyping was conducted with a multiplex amplification refractory mutation system polymerase chain reaction using the method reported previously [27].

Statistics

Statistical analyses were performed using the t test, ANOVA, and χ^2 test in SPSS 20.0. Comparisons were made using Pearson's χ^2 or Fisher's exact test when appropriate. Multivariate regression analysis was performed to exclude the interference of CAG repeat length on AAO. We performed separate analysis to control the bias caused by sex difference. P <0.05 was considered statistically significant.

Results

Clinical Characteristics of Participants

Two hundred and twenty-three unrelated (118 male/105 female) HD patients with genetic confirmation were recruited in this study. They were from different families, so the bias created by duplications of *APOE* alleles in family members was excluded. Among them, 18 lacked details of the medical history such as initial symptoms and AAO, and 13 were without *APOE* genotype data including two who also lacked medical history information. Thus, only 205 patients had clear initial symptoms. Among them, 184 (89.8%) presented with movement symptoms first, 10 (4.9%) with psychiatric symptoms, and 8 (3.9%) with cognitive decline. In addition, one person presented with seizure as the initial symptom. Two had concurrent psychiatric and cognitive symptoms. Thirteen cases were juvenile HD (JHD) and the remaining 192 were adult-onset HD. Among the 13 JHD cases, the initial symptoms were motor disorders (n = 10), cognitive impairment (n = 2) and seizure (n = 1).

Frequency Distributions of *APOE* Genotypes in HD Patients and Controls

The 210 patients with *APOE* genotype data were analyzed for the *APOE* genotype frequency. The distributions of *APOE* alleles were significantly different between HD and control populations (Table 1). The E4 allele had a frequency of 7.1% in the HD cohort, which is less than the 12.0% in the control population (P = 0.004). The E3 allele had a higher frequency of 89.0% in the HD patients than the 81.7% in the controls (P = 0.0002). The distributions of *APOE* genotypes were also statistically different. The E3E3 genotype was significantly more frequent (80.0%) in the HD cohort than in the controls (69.1%) (P = 0.001), while the E3E4 genotype was less frequent (11.9%) in the HD population than in the control population (18.0%) (P = 0.031). After stratifying the allele distributions by sex, the results for *APOE* alleles were consistent with those before stratification. However, significant differences in the E3E3 and E3E4 genotypes remained in the female group but disappeared in the male group (Table 1).

Influence of CAG or Sex on AAO

As 18 HD patients lacked AAO information, 205 with both AAO and CAG data were included in the analysis. The AAO here refers to the onset age at motor symptoms. The mean and median AAO were 40.0 ± 11.8 years (range 4–70) and 40 years. And the mean and median expanded CAG repeat number were 46.4 ± 7.8 (range 39–104) and 44. The distributions of AAO and CAG are shown in Figs. 1 and 2, respectively. There was a negative correlation between AAO and CAG repeat length (Fig. 3). Using exponential regression analysis, 73% of the variance in AAO can be explained by the expanded CAG length (R^2= 0.73). To investigate the influence of sex on AAO, we divided the cohort into male (n = 106) and female (n = 99) groups. However, there was no significant difference in AAO between the two groups (male: 39.8 ± 12.4 years, range 8–70 years; female: 40.1 ± 11.1 years, range 4–60 years; P = 0.88).

APOE Did Not Affect Initial Symptoms or AAO in HD Patients

To test whether the *APOE* genotype affects initial symptoms, we classified patients into motor-onset and non-motor-onset groups. Among the 205 patients with clear initial symptoms, 10 (including one with JHD) lacked *APOE* genotype information were excluded. Thus, 195 patients were classified into a motor onset group (n = 175) and a non-motor onset group (n = 20). To minimize the influence of CAG repeat length on initial symptoms, the patients were further divided into two narrow CAG-range subgroups according to the median CAG (44): CAG ≤44 and CAG >44. There was no significant difference in the distributions of *APOE* alleles or genotypes between the motor and non-motor groups either in full-range CAG or in the narrow CAG-ranges (Table 2).

JHD is characterized by atypical symptoms such as epilepsy, bradykinesia, dystonia, and myoclonus, which are quite different from adult-onset HD [28–30]. Thus, we further divided the 195 patients into a JHD group (n = 12) and an adult-onset group (n = 183). Since the number of JHD cases was small (10 motor onset and 2 non motor onset), we only analyzed the frequency distributions of *APOE* genotypes in adult-onset group and found the distributions were no different (Table 2).

The *APOE* E4 allele is thought to be associated with cognitive decline in many neurodegenerative diseases. As the number of patients with an initial symptom of cognitive

Table 1 Frequency distributions of *APOE* genotypes in HD patients and controls.

Groups	Allele			Genotype					
	E2 (%)	E3 (%)	E4 (%)	E2E2 (%)	E2E3 (%)	E2E4 (%)	E3E3 (%)	E3E4 (%)	E4E4(%)
HD patients	16 (3.8)	374 (89.0)	30 (7.1)	1 (0.5)	13 (6.2)	1 (0.5)	168 (80.0)	25 (11.9)	2(1.0)
Controls	156 (6.3)	2028 (81.7)	298 (12.0)	13 (1.0)	89 (7.2)	41 (3.3)	858 (69.1)	223 (18.0)	17(1.4)
P value	0.047*	0.0002*	0.004*	0.707	0.607	0.024*	0.001*	0.031*	1.000
HD females	8 (4.0)	178 (89.9)	12 (6.1)	0 (0.0)	7 (7.1)	1 (1.0)	82 (82.8)	7 (7.1)	2(2.0)
Control females	96 (6.7)	1169 (82.0)	161 (11.3)	9 (1.3)	55 (7.7)	23 (3.2)	496 (69.6)	122 (17.1)	8(1.1)
P value	0.147	0.005*	0.025*	0.610	0.821	0.344	0.006*	0.010*	0.349
HD males	8 (3.6)	196 (88.3)	18 (8.1)	1 (0.9)	6 (5.4)	0 (0.0)	86 (77.5)	18 (16.2)	0(0.0)
Control males	60 (5.7)	859 (81.3)	137 (13.0)	4 (0.8)	34 (6.4)	18 (3.4)	362 (68.6)	101 (19.1)	9(1.7)
P value	0.210	0.013*	0.043*	1.000	0.683	0.054	0.062	0.474	0.371

*P value < 0.05.

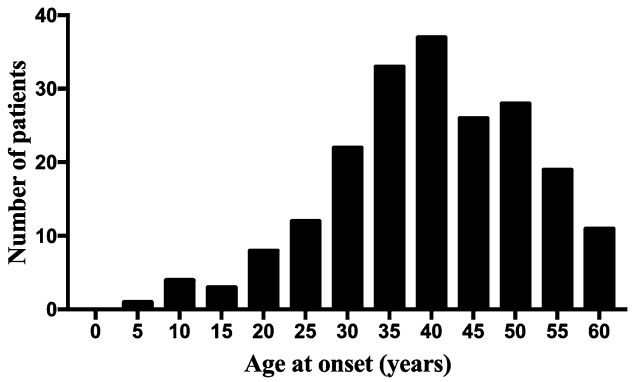

Fig. 1 Frequency distribution of AAO in patients with HD.

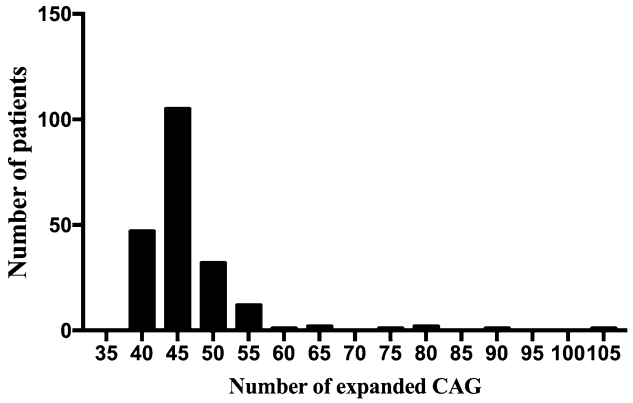

Fig. 2 Frequency distribution of expanded CAG repeat length in *HTT* in patients with HD.

decline was too small to be analyzed ($n = 8$), we just presented their genetic features (CAG repeat lengths and *APOE* genotypes) in Table S1. Surprisingly, they were all of the E3E3 genotype.

To determine whether *APOE* can modify the AAO of HD, we divided these 195 patients into E2E3, E3E3, and

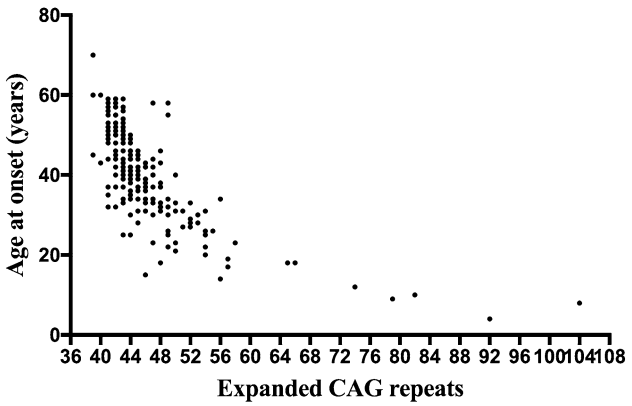

Fig. 3 Relationship between the expanded CAG repeat length and AAO. The CAG repeat number explained ~73% of the variance in AAO.

E3E4 groups, as the numbers of patients with E2E2 ($n = 1$), E2E4 ($n = 1$), and E4E4 ($n = 2$) were too small to be analyzed. The mean AAOs for the E2E3, E3E3, and E3E4 groups were 42.9 ± 8.9 years ($n = 12$), 39.5 ± 12.0 years ($n = 156$), and 42.4 ± 12.1 years ($n = 23$), respectively. No statistical difference was found in AAO between the three *APOE* genotypes ($P = 0.38$). When stratified by sex, the results were also negative (female: $P = 0.14$; male: $P = 0.87$). In addition, patients were divided into E4 carrier and E4 non-carrier groups. The mean AAOs were 42.8 ± 11.6 years ($n = 26$) and 39.8 ± 11.8 years ($n = 169$), respectively. However, there was no difference in AAO between E4 carriers and non-carriers either in the total cohort ($P = 0.22$) or in males ($P = 0.67$) or females ($P = 0.14$) (Table 3).

The correlation between CAG repeat length and AAO differs between JHD and adult-onset HD [31]. Hence, we also compared the AAO in the JHD and adult-onset HD groups. Among the 12 JHD patients, 11 had the E3E3

Table 2 Relationship between *APOE* genotypes and initial symptoms of HD.

Groups	Alleles			Genotype					
	E2 (%)	E3 (%)	E4 (%)	E2E2 (%)	E2E3 (%)	E2E4 (%)	E3E3 (%)	E3E4 (%)	E4E4 (%)
Total									
Motor	14 (4.0)	309 (88.3)	27 (7.7)	1 (0.6)	11 (6.3)	1 (0.6)	138 (78.9)	22 (12.6)	2 (1.1)
Non-motor	1 (2.5)	38 (95)	1 (2.5)	0 (0.0)	1 (5.0)	0 (0.0)	18 (90.0)	1 (5.0)	0 (0.0)
P value	1.000	0.287	0.338	1.000	1.000	1.000	0.376	0.478	1.000
CAG $\leq$ 44									
Motor	8 (4.5)	154 (86.4)	16 (9.0)	1 (1.1)	6 (6.7)	0 (0.0)	67 (75.3)	14 (15.7)	1 (1.1)
Non-motor	1 (3.8)	25 (96.2)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	12 (92.3)	0 (0.0)	0 (0.0)
P value	1.000	0.212	0.232	1.000	1.000	1.000	0.288	0.206	1.000
CAG > 44									
Motor	6 (3.5)	155 (90.1)	11 (6.4)	0 (0.0)	5 (5.8)	1 (1.2)	71 (82.6)	8 (9.3)	1 (1.2)
Non-motor	0 (0.0)	13 (92.9)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	6 (85.7)	1 (14.3)	0 (0.0)
P value	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.522	1.000
Adult-onset									
Motor	14 (4.2)	290 (87.9)	26 (7.9)	1 (0.6)	11 (6.7)	1 (0.6)	129 (78.2)	21 (12.7)	2 (1.2)
Non-motor	1 (2.8)	34 (94.4)	1 (2.8)	0 (0.0)	1 (5.6)	0 (0.0)	16 (88.9)	1 (5.6)	0 (0.0)
P value	1.000	0.405	0.497	1.000	1.000	1.000	0.373	0.701	1.000

Table 3 Comparison of AAO between different *APOE* genotype subgroups of HD.

Groups	Genotype				
	E2E3	E3E3	E3E4	E4+	E4-
Total HD					
Mean AAO (SD) (years)	42.9 (8.9)	39.5 (12.0)	42.4 (12.1)	42.8 (11.6)	39.8 (11.8)
Number	12	156	23	26	169
P value	0.38			0.22	
Females HD					
Mean AAO (SD) (years)	46.3 (8.2)	39.2 (11.7)	45.1 (7.3)	45.4 (6.6)	39.8 (11.6)
Number	7	77	7	10	84
P value	0.14			0.67	
Males HD					
Mean AAO (SD) (years)	38.2 (8.2)	39.8 (12.3)	41.2(13.8)	41.2 (13.8)	39.8 (12.0)
Number	5	79	15	16	85
P value	0.87			0.14	
Adult-onset HD					
Mean AAO (SD) (years)	42.9 (8.9)	41.4 (10.1)	43.9 (10.1)	44.2 (9.6)	41.6 (10.0)
Number	12	145	22	15	168
P value	0.51			0.33	

genotype with a mean AAO of 14.9 $\pm$ 4.9 years, and one had the E3E4 genotype with an AAO of 10 years. To avoid the interference caused by JHD, we only analyzed AAO in the adult-onset group and the results showed no significant difference in AAO between the *APOE* genotype subgroups (Table 3).

Although we divided patients into two narrow CAG-range groups (group one and group two) or two different onset groups (JHD and adult-onset HD) to minimize the interference of the CAG repeat length, we still used multivariate regression analysis for further confirmation. We set the AAO as a dependent variable, with CAG repeat length and *APOE* genotypes as independent variables. There was no correlation between the *APOE* genotypes and AAO (E2E3 *vs* E3E3, $P = 0.72$; E2E3 *vs* E3E4, $P = 0.64$; E3E3 *vs* E3E4, $P = 0.15$). Moreover, in groups with or

without E4, the result was also negative ($P = 0.13$). When stratified by sex, no statistical difference was found in any group: male (E2E3 *vs* E3E3, $P = 0.24$; E2E3 *vs* E3E4, $P = 0.19$; E3E3 *vs* E3E4, $P = 0.19$; E4+ *vs* E4–, $P = 0.17$); female (E2E3 *vs* E3E3, $P = 0.18$; E2E3 *vs* E3E4, $P = 0.82$; E3E3 *vs* E3E4, $P = 0.52$; E4+ *vs* E4–, $P = 0.40$).

Discussion

The *APOE* E4 allele is thought to increase the risk of AD, DLB [20], and frontotemporal dementia [32]. The frequencies of the E2, E3, and E4 alleles in the general population are 8.4%, 77.9%, and 13.7%, respectively, according to a meta-analysis covering 8607 controls [33]. However, in AD patients, the E4 allele frequency is dramatically higher at ~40% [33]. In our HD cohort, the E4 allele was 7.1%, less frequent than the 12.0% in our control population and 13.7% reported in the general population [33]. Of note, the frequency of the E4 allele varies among different HD patient populations: Greek, 10.8% [23], Welsh, 13.5% [24], and German, 15.1% [34]. This may be caused by ethnic differences in genetic backgrounds as the frequency of the *APOE* E4 allele in the general population also varies, with 7.4% in Japanese and 29.3% in Sudanese [13].

In our HD cohort, 73% of the variation in AAO could be explained by the number of CAG repeats, indicating that other modifiers might have an influence on AAO, such as environmental factors, the sex of the transmitting parent, and genetic *cis*-elements [35, 36]. Previous studies have investigated the influence of *APOE* on AAO in HD patients, but their conclusions are controversial. In one study, the E4 allele was thought to delay the AAO in HD patients [23]. In another study, the results showed that the E2E3 genotype was significantly associated with an earlier AAO in male HD patients [24]. Both studies enrolled patients with large ranges of CAG repeats (40–57 CAG, $n = 60$ and 38–67 CAG, $n = 138$, respectively). However, in an HD cohort ($n = 167$) with a very narrow range of CAG repeats (41–45), the *APOE* genotypes did not influence the AAO in HD patients [34]. In our HD cohort, we also did not find a correlation between *APOE* genotypes and HD phenotypes.

There are some limitations in our study. First, the sample size was comparatively small, especially patients with cognitive impairment and behavior dysfunction. The numbers of E4 carriers and JHD patients were also limited. Second, as one report on motor neuron disease showed that the E4 allele was significantly more common in patients with bulbar onset than in those with limb onset, the E4 allele may influence the pattern of motor neuron loss in a way unrelated to the deposition of Aβ [37]. Accordingly, the relationship of *APOE* genotypes and body part (head, limbs, or trunk) where chorea starts in HD patients may be worth studying.

In summary, our study is the first to demonstrate that *APOE* genotypes do not influence the HD phenotypes, especially in adult-onset HD of Han Chinese origin. Additional studies are needed to elucidate other genetic modifiers playing in the pathogenesis of HD.

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Conflict of interest All authors claim that there are no conflicts of interest.

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