The Role of Apolipoprotein E as a Risk Factor for an Earlier Age at Onset for Machado-Joseph Disease Is Doubtful

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Abstract

Machado-Joseph disease (MJD) is an inherited neurodegenerative disease caused by an expanded CAG repeat in the ATXN3 gene. Although the principal genetic determinant of the age at onset (AAO) is the length of the expanded CAG repeat, the additional genetic contribution of MJD toward the AAO has mostly not yet been clarified. It was recently suggested in two independent studies that apolipoprotein E (APOE) might be associated with AAO variability in MJD patients. To identify the potential modifier effect of APOE polymorphisms on the AAO of MJD patients, 403 patients with MJD (confirmed by molecular tests) from eastern and southeastern China were enrolled in the present study. CAG repeats in the ATXN3 and APOE polymorphisms were genotyped. Data were analyzed using a statistical package. No contribution of APOE polymorphisms to the variance in disease onset was observed using ANCOVA (F = 0.183, P = 0.947). However, significant effects on the AAO of MJD were found for the normal ATXN3 allele and for the interaction of mutant and normal ATXN3 alleles in a multiple linear regression model (P = 0.043 and P = 0.035, respectively). Our study does not support a role for APOE as a genetic modifier of the AAO of MJD. Additionally, our study presents evidence that the normal ATXN3 allele and its interaction with mutant alleles contribute toward AAO variance in MJD patients.

Introduction

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder characterized by cerebellar ataxia associated with marked phenotypic heterogeneity [1]. The disease usually starts during adulthood, but the age at onset (AAO) ranges from adolescence to old age [2]. MJD is caused by a CAG repeat expansion in exon 10 of the ATXN3 gene that translates to an elongated polyglutamine tract in the ataxin-3 protein [3]. Similar to several other polyglutamine disorders, the AAO for MJD patients is not completely determined by the expanded CAG repeat. Previous studies have demonstrated that the length of the mutant CAG repeat explains approximately 45–87% of the total AAO variance of MJD [4–6]. Familial factors are responsible for some of the residual variance [5,7], indicating that modifier genes may play a role in the AAO variance. The identification and characterization of these modifiers offer an important opportunity to better understand the biological mechanisms involved in the disease, improving consultation services for pre-symptomatic individuals.

The human apolipoprotein E (APOE) gene is polymorphic, with three major alleles (ε2, ε3, and ε4) that differ from each other at two crucial non-synonymous sites [8,9]. Therefore, the corresponding three isoforms, E2, E3, and E4, ultimately possess distinct structural and functional properties [10]. The APOE polymorphisms have been investigated in a number of neurodegenerative diseases with respect to risk, progression, and AAO [11–14]. In particular, the association of APOE with the AAO for Huntington’s disease (HD) was reported in several studies, but the results were inconsistent [15–19].

Recently, two independent groups reported an association of the APOE ε2 allele with an earlier AAO for MJD in small samples (fewer than 200 patients) [20,21]. To further investigate this issue, we collected a large cohort of MJD patients (more than 400 individuals) from eastern and southeastern China and then analyzed the association of APOE polymorphisms with the AAO.
Materials and Methods

MJD patients

In the present study, 403 patients from 362 unrelated families with molecular confirmation of MJD were enrolled. The patients were surveyed between February 2005 and March 2013, and they were all from eastern and southeastern China. Each patient gave informed consent, and the local ethics committees approved the study protocol. AAO was defined as the age at which the first symptoms of ataxia occurred. Whenever possible, the AAO was corroborated by close relatives or care providers.

Molecular analyses

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following standard procedures. Molecular analysis of ATXN3 was performed as previously reported [22], and the lengths of the expanded and normal CAG repeats were determined using Sanger sequencing. The APOE polymorphisms (SNP combinations of rs429358 and rs7412) were determined using fluorescence-based polymerase chain reaction-restriction fragment length polymorphism analysis. Primer pairs were designed to genotype rs429358 (fluorescence-labeled forward primer 5'-[FAM] AGG GGC CTG ATG GAC GAG AC-3' and reverse primer 5'- GCC CCG GCC TGG TAC ACT-3') and rs7412 (fluorescence-labeled forward primer 5'-[FAM] GGC GGC GAC ATG GAC GAC-3' and reverse primer 5'-GCC CCG GCC TGG TAC ACT-3'). PCR reactions for the two SNPs were each performed in a 10 μL reaction mixture containing 1x GC buffer I, 2 mM MgCl2, 0.2 mM dNTP, 2 μM primers, 1 U Hotstar Taq polymerase (Qiagen, Hilden, Germany), and 30 ng template DNA, using the following cycling conditions: an initial denaturation at 95°C for 15 min, followed by 11 cycles of 94°C for 20 s, 65°C (0.5°C decrease per cycle) for 40 s, 72°C for 1.5 min, then another 24 cycles of 94°C for 20 s, 59°C for 30 s, and 72°C for 1.5 min, and a final extension step at 72°C for 2 min. The amplification products were then incubated with the endonucleases AluIII (1 U) and HaeII (1 U) (New England Biolabs, Beverly, MA, USA), respectively, according to the manufacturer's recommendations. Finally, the corresponding digestion products and a pair of control fragments were collectively detected using automated capillary electrophoresis in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems).

Statistical analyses

All statistical analyses were performed using commercially available software (SPSS, version 17.0, downloaded for free from http://www.stathome.cn/html/down/ruanjian/2009/0601/435.html). A p value <0.05 (two-tailed) was considered to be statistically significant. The variability in the AAO attributable to the expanded CAG repeat was calculated using linear regression. Either Fisher’s exact test or a chi-square test was used to compare the distribution frequency of APOE genotypes or alleles between our cohort and the cohorts reported by Bettencourt et al. [20] and Peng et al. [21]. Differences in the AAO according to the APOE genotype and gender of the patient were examined using a two-tailed t-test or an ANCOVA, adjusted for the expanded ATXN3 allele. Multiple linear regressions were used to calculate the effect of several factors on the AAO: the number of CAG repeats in the expanded and normal ATXN3 alleles, their interaction, the APOE status, and the patient gender. To further validate the association between APOE polymorphisms and the AAO in our sample of 403 MJD patients, they were sorted according to gender. Differences in the AAO according to the APOE genotype of each group were examined using ANCOVA after adjusting for the expanded ATXN3 allele. Furthermore, to reduce potential deviation encountered while defining the AAO, differences in the AAO according to the APOE genotype in 225 MJD patients with a shorter duration of disease (less than or equal to 3 years) were examined using ANCOVA after adjusting for the expanded ATXN3 allele.

Results

The average AAO (±SE) of this cohort of 403 MJD patients (211 males and 192 females) was 36.28 (±0.56) years (range: 10–72 years). The average CAG repeat size (±SE) was 75.90 (±0.186) repeats (range: 53–87 repeats). A chromatogram of an MJD patient with 14/86 CAG repeats is shown in Figure S1. The capillary electrophoresis analysis of APOE polymorphisms is shown in Figure S2.

As expected, the number of expanded CAG repeats was negatively associated with the AAO (Pearson R2 = 0.659, p = 0.000, Figure S3), in accordance with previous studies [4–6]. The characteristics of this patient cohort are presented in Table 1. There was no significant difference in the genotypic or allelic distributions of APOE between the present cohort and the cohorts reported by Bettencourt et al. [20] (genotype: p = 0.789; allele: p = 0.501) and Peng et al. [21] (genotype: p = 0.44; allele: p = 0.872). Patients carrying different APOE genotypes showed no differences in the AAO adjusted for the expanded ATXN3 allele (ANCOVA, F = 0.18, p = 0.9474). Furthermore, the gender of the patients did not affect the AAO according to either a two-tailed t-test (p = 0.087) or an ANCOVA when using the number of expanded CAG repeats as a covariate (F = 0.435, p = 0.510).

Compared to the linear model that included only the number of CAG repeats in the abnormal allele, multiple linear regression analysis increased the predictive value of the age at onset by an additional 0.7% (from 63.9% to 66.6% of variance in the AAO). From this model, we found a significant contribution to the variance in the AAO from the number of CAG repeats in the expanded (p = 0.000) and normal alleles (p = 0.043), as well as from their interaction (p = 0.035). Both the APOE genotype and the patient gender failed to show significant effects on the AAO in this model (p = 0.892 and p = 0.512, respectively), in agreement with the results of the ANCOVA.

When we sorted the MJD patients according to gender to validate the association between APOE polymorphisms and the AAO, the results indicated that there was no sex specific influence of APOE genotype (ANCOVA, male: p = 0.915; female: p = 0.849); Furthermore, our evaluation of the 225 MJD patients with a shorter duration of disease indicated that there were no differences in the AAO according to the APOE genotype (ANCOVA, p = 0.627).

Discussion

The underlying pathophysiology of MJD is complex and remains unclear. Association studies of candidate genes assumed to be involved in MJD pathogenesis provide a good opportunity to obtain useful clues for understanding MJD pathogenesis. It is well established that APOE plays an important role in mediating cholesterol metabolism and regulating neural growth, regeneration, and repair in the central nervous system. We were therefore motivated to investigate the effect of APOE polymorphisms on the AAO in a large cohort of Chinese Han MJD patients. Unfortunately, our study failed to show a significant association between the APOE ε2 allele and the AAO for MJD, although we analyzed a cohort of patients larger than those of previous studies.
To minimize the influences of gender and disease duration on the results, all patients were further stratified by sex and disease duration and then re-examined; however, the results were still negative. There are several possible explanations for this discrepancy.

First, the sizes of the studied samples vary. The sample size of the present study is twice as large as the sample sizes of the previous studies [20,21]. Similarly, among association studies of APOE that indicated an earlier AAO for HD, the sample sizes were larger in the negative reports [17–19] than in the positive reports [15,16]. It should be noted that only prospective studies are capable of obtaining precise data with respect to the AAO. Despite this limitation, we can achieve a decent approximation toward AAO variance in MJD patients.

Second, the genetic heterogeneity across populations could account for the discrepancy, including differences in genotypic distributions and the underlying linkage disequilibrium (LD) patterns. Compared with the findings of Bettencourt et al. [20] and Peng et al. [21], we did not observe a significant difference in the genotypic distributions of APOE. Therefore, the lack of association with the APOE e2 allele may be due to different LD structures in the region surrounding APOE across populations. In other words, there may be other functional polymorphisms that lead to early AAO in strong LD with the APOE e2/e3 genotype in the patients examined by Bettencourt et al. [20] and Peng et al. [21], whereas a similar LD pattern simply does not exist in our cohort from the Han population of eastern and southeastern China.

Third, because genetic interaction with various environments may produce distinct phenotypes, the standard of living and other environmental factors may also modulate the effects of APOE polymorphisms on the AAO of MJD patients. Additionally, the present results of the multiple regression analysis suggested that the normal ATXN3 allele and the interaction of the mutant and normal ATXN3 alleles contribute to the AAO variance in MJD. Similarly, several studies reported the same results in MJD [6,23] and HD [24,25]. It was observed that the processes of aggregation, nucleation, and cytotoxicity of mutational polyglutamine proteins were exacerbated by their normal counterparts in a Drosophila model [26]. Specific analyses of the underlying mechanism of this interaction in MJD requires further elucidation.

In conclusion, our study casts doubt on the role of APOE as a risk factor for an earlier age at onset for MJD. The normal ATXN3 allele and its interaction with mutant alleles contribute toward AAO variance in MJD patients.

Supporting Information

Figure S1 Chromatogram of MJD patients with CAG repeats of 14/86. Normal CAG repeat expansion is indicated by a thick black arrow, whereas the abnormal CAG repeat sequence is colored in gray.

Figure S2 Capillary electrophoresis analysis of the genotypes of APOE*. *The APOE polymorphisms are the SNP combinations rs429358 and rs7412. The amplification products for rs429358 that could be cleaved by the AluIII endonuclease into fluorescently labeled 153-bp and non-fluorescently labeled 164-bp fragments indicated allele T (capillary electrophoresis analysis revealed one peak at 153 bp), whereas the products that could not be cleaved showed a 317-bp peak in the capillary electrophoresis analysis and indicated allele C. Similarly, the amplification products for rs7914 that could be cleaved by the HaeII endonuclease into fluorescently labeled 162-bp and non-fluorescently labeled 23-bp fragments indicated allele C (capillary electrophoresis analysis revealed one peak at 162 bp), whereas those that could not be cleaved showed a 185-bp peak in the capillary electrophoresis analysis and indicated allele T.

Figure S3 Negative correlation between the age at onset and the number of expanded CAG repeats in MJD.

Table 1. Characteristics of the studied series of MJD patients.

| Variables                        | APOE Genotype | Total |
|----------------------------------|---------------|-------|
|                                  | e2/e3 | e3/e3 | e3/e4 | e2/e4 | e4/e4 |

| Number of patients (%) | 53 (13.2) | 263 (65.3) | 75 (18.6) | 9 (2.2) | 3 (0.7) | 403 (100) |
|------------------------|-----------|------------|-----------|---------|---------|-----------|
| Gender, No. (%)        | Male      | 33 (15.6)  | 130 (61.6) | 40 (19.0) | 6 (2.8) | 2 (0.9) | 211 (100) |
|                        | Female    | 20 (10.4)  | 133 (69.3) | 35 (18.2) | 3 (1.6) | 1 (0.5) | 192 (100) |
| Age at onset, y         | Mean ± SE [range] | 34.14 (1.40) | 36.12 (0.67) | 38.32 (1.56) | 35.89 (2.53) | 39.17 (1.88) | 36.28 (0.56) |
|                         | [10–54] | [14–59] | [13–72] | [24–46] | [36–42] | [10–72] |
| Adjusted onset ± SE     | 35.90 (0.91) | 36.29 (0.41) | 36.51 (0.76) | 35.59 (2.20) | 38.59 (3.81) |
| CAG repeat length, No.  | Normal, mean (SE) [range] | 18.92 (0.92) | 19.86 (0.43) | 19.59 (0.82) | 22.89 (2.60) | 14.00 (0.00) | 19.71 (0.346) |
|                         | [14–34] | [14–38] | [14–37] | [14–35] | 14 | [14–38] |
|                         | Expanded, mean (SE) [range] | 76.62 (0.48) | 75.97 (0.21) | 75.16 (0.57) | 75.78 (0.46) | 75.67 (0.88) | 75.90 (0.186) |
|                         | [67–87] | [60–84] | [53–86] | [74–79] | [74–77] | [53–87] |

*Adjusted for the average expanded CAG repeat length in the studied series of MJD patients.

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The regression line is $Y = -2.4X + 222$ (Y: age of onset, X: number of expanded CAG repeats).

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References

1. Takiyama Y, Oyanagi S, Kawashima S, Sakamoto H, Saito K, et al. (1994) A clinical and pathologic study of a large Japanese family with Machado-Joseph disease tightly linked to the DNA markers on chromosome 14q. Neurology 44: 1302–1308.
2. Durr A (2010) Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet Neurol 9: 883–894.
3. Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, et al. (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q22.1. Nat Genet 8: 221–228.
4. Maciel P, Gaspar C, DeStefano AL, Silveira I, Coutinho P, et al. (1994) Correlation between CAG repeat length and clinical features in Machado-Joseph disease. Am J Hum Genet 54: 52–61.
5. van de Warrenburg BP, Hendriks H, Durr A, van Zuijlen MC, Stevanin G, et al. (2005) Age at onset variance analysis in spinocerebellar ataxias: a study in a Dutch-French cohort. Ann Neurol 57: 505–512.
6. França MC Jr, Emmer VE, D’Abreu A, Maurer-Morelli CV, Secolin R, et al. (2012) Normal ATXN3 Allele but Not CHIP Polymorphisms Modulates Age at Onset in Machado-Joseph Disease. Front Neurol 3: 164.
7. DeStefano AL, Cupples LA, Maciel P, Gaspar C, Rachmany J, et al. (1996) A familial factor independent of CAG repeat length influences age at onset of Machado-Joseph disease. Am J Hum Genet 59: 119–127.
8. Lin-Lee YC, Kao FT, Chen Z, Chan I. (1985) Apolipoprotein E gene mapping and expression: localization of the structural gene to human chromosome 19 and expression of APOE mRNA in lipoprotein- and non-lipoprotein-producing tissues. Biochemistry 24: 3751–3756.
9. Weisgraber KH, Rall SC Jr, Mahley RW. (1981) Human E apoprotein heterogeneity: Cysteine-arginine interchanges in the amino acid sequence of the apoE isoforms. J Biol Chem 256: 9077–9083.
10. Hauser PS, Narayanaswami V, Ryan RO. (2011) Apolipoprotein E: from lipid transport to neurobiology. Prog Lipid Res 50: 62–74.
11. Kehoe P, Krawczak M, Harper PS, Owen MJ, Jones AL. (1999) Age of onset in Huntington disease: sex specific influence of apolipoprotein E genotype and normal CAG repeat length. J Med Genet 36: 108–111.
12. Panas M, Avramopoulos D, Karadima G, Petersen MB, Vassilopoulos D. (1999) Apolipoprotein E and presenilin-1 genotypes in Huntington’s disease. J Neurol 246: 574–577.
13. Kuhntmeiner DC, Leggo J, Chiano M, Dodge A, Norbury G, et al. (1997) Genotypes at the GluR6 kainate receptor locus are associated with variation in the age of onset of Huntington disease. Proc Natl Acad Sci U S A 94: 3872–3876.
14. Saft C, Andrich JE, Brune N, Gencik M, Kraus PH, et al. (2004) Apolipoprotein E genotypes do not influence the age of onset in Huntington’s disease. J Neurol Neurosurg Psychiatry 75: 1692–6.
15. Andresen JM, Gayan J, Cheriny SS, Brocklebank D, Alkorta-Aranburu, et al. (2007) Replication of twelve association studies for Huntington’s disease residual age of onset in large Venezuelan kindreds. J Med Genet 44: 44–50.
16. Bettencourt C, Raposo M, Czymrok T, Santos C, et al. (2011) The APOE ε2 allele increases the risk of earlier age at onset in Machado-Joseph disease. Arch Neurol 68: 1580–1583.
17. Peng H, Wang C, Chen Z, Sun Z, Jiao B, et al. (2014) The APOE ε2 allele may decrease the age at onset in patients with spinocerebellar ataxia type 3 or Machado-Joseph disease from the Chinese Han population. Neurobiol Aging 22:pi: S0979-638X(14)00274-00277.
18. Dai SR, Shi SS, Wu JJ, Wang N, Zhao GX, et al. (2010) High frequency of Machado-Joseph disease identified in southeastern Chinese kindreds with spinocerebellar ataxia. BMC Med Genet 11: 47.
19. Durr A, Svanesin G, Cancel G, Dusckaerts C, Abbas N, Didierjean O, et al. (1996) Spinocerebellar ataxia type 3 and Machado-Joseph disease: clinical, molecular, and neuropathological features. Ann Neurol 39: 490–499.
20. Djousse L, Knowldon B, Hayden M, Ahmqsit EW, Brinkman R, et al. (2003) Interaction of normal and expanded CAG repeat sizes influences age at onset of Huntington disease. Am J Med Genet A 119A: 279–282.
21. Aziz NA, Jurgens CK, Landwehrmeyer GB, EHDD Registry Study Group, van Roon-Mom WM, et al. (2011) Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease. Neurology 73: 1210–1215.
22. Slepek N, Bhattacharyya AM, Jackson GR, Steffan JS, Marsh JL, et al. (2006) Normal-repeat-length polyglutamine peptides accelerate aggregation nucleation and cytotoxicity of expanded polyglutamine proteins. Proc Natl Acad Sci U S A 103: 14367–14372.

Author Contributions

Conceived and designed the experiments: SRG ZYW. Performed the experiments: QZ WN YD NW SRG ZYW. Analyzed the data: QZ SRG. Contributed reagents/materials/analysis tools: QZ WN YD NW SRG ZYW. Wrote the paper: QZ WN. Critical revision of the manuscript for important intellectual content: SRG ZYW.

APOE and Earlier AAO of MJD