J Neurol (1999) 246 : 574–577

ORIGINAL COMMUNICATION

Marios Panas
Dimitrios Avramopoulos
Georgia Karadima
Michael B. Petersen
Demetrios Vassilopoulos

Apolipoprotein E and presenilin-1 genotypes in Huntington's disease

Received: 13 May 1998
Received in revised form: 9 December 1998
Accepted: 31 December 1998

M. Panas · D. Avramopoulos
D. Vassilopoulos (✉)
Department of Neurology
of Athens National University.
74 Vas. Sophias Av. GR-11528,
Athens, Greece
Tel.: +30-1-7244917, Fax: +30-1-7244917

G. Karadima · M. B. Petersen
Institute of Child Health.
Aghia Sophia Childrens Hospital,
Athens, Greece

Abstract Huntington's disease (HD) is an autosomal dominant degenerative disease of the central nervous system manifested by involuntary movements (chorea), psychiatric manifestations, and cognitive impairment with a variable age at onset. This variability is mainly attributed to genetic factors. The so-called aging genes [e.g., those for apolipoprotein E (*APOE*) and presenilin-1 (PS-1) have been implicated in determining the age at onset of Alzheimer's disease, a disease sharing common clinical features with HD. In 60 unrelated patients suffering from HD (mean age at onset 40.1 years, range 20–65) we determined number of CAG repeats and the distribution of the *APOE* alleles (ε2, ε3, ε4) and *PS-1* alleles. The results showed that: (a) The age at onset was higher in the group of patients with the ε4 allele (51.6 vs. 38.0 $P < 0.002$), (b) The correlation between the age at onset and the number of CAG repeats was strong in patients with the ε3/ε3 genotype while it was not detected in patients with ε3/ε4 genotype. (c) No correlation was found between age at onset and *PS-1* alleles. In conclusion, *APOE* seems to be a significant factor influencing the age at onset of Huntington's disease.

Key words Apolipoprotein E · Presenilin-1 · Huntington's disease

Introduction

Huntington's disease (HD) is a progressive degenerative disorder clinically characterized by a triad of symptoms: involuntary movements (chorea), psychiatric manifestations, and cognitive impairment with a variable age at onset (average 35–40 years [12]). The best documented factor determining the age at onset is a genetic defect: an expanded CAG trinucleotide repeat in the IT15 gene on chromosome 4 [14]. In individuals not affected with HD the number of copies of the CAG trinucleotide varies between 9 and 39. In HD patients the number of repeats is 36 or higher, and up to 120 copies have been reported [17, 21]. A strong correlation between the age at onset and the number of CAG repeats has been well documented [22, 24]. However, it appears that the number of CAG repeats is not the only factor determining the age at onset of the disease, being responsible only for 50% of the total variance [1, 22, 24]. Other factors have been proposed as playing an additional role in influencing the age at onset [8–11]. Of all possible factors, the genes for apolipoprotein E (*APOE*) and presenilin 1 (*PS-1*) have been shown to play a significant role in other disorders in which age is an important factor, such as Alzheimer's disease [3, 4, 6, 23] and Down's syndrome [2].

We examined the possibility of a correlation between the age at onset of HD and the allelic variations in the *APOE* and *PS-1* genes.

Materials and methods

Patients

We used DNA from 60 unrelated patients (28 men, 32 women) previously diagnosed both clinically and by DNA analysis in our

laboratory as suffering from HD; all patients had given their informed consent for genotyping with *APOE* and *PS-1*. The age at onset of the disease was defined as the age at which movement disorder (chorea, rigidity, or clear voluntary movement impairment) was first observed in the patient as documented by the interview and review of the records, blind to the DNA results. Our control group consisted of 45 random voluntary blood donors. Our results were also compared to data from a population survey of Greeks on the distribution of *APOE* alleles [20].

DNA was extracted from blood lymphocytes using a salt extraction method as previously described [16]. Genotyping of the polymorphic *APOE* alleles was performed after polymerase chain reaction amplification of genomic DNA using primers and reaction conditions as described by Wenham et al. [26]. In the thermal reactor (MJ Research) an initial denaturation at 94 °C for 5 min was followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 30 s, and final extension at 72 °C for 10 min. The 25-µl amplification product was then digested with 10 U restriction enzyme *Hha*I at 37 °C for at least 3 h. Restriction fragments were separated by electrophoresis on a 3% NuSieve-1% agarose gel and visualized by ethidium bromide staining [2].

Genotyping of the polymorphic *PS-1* alleles was performed after polymerase chain reaction amplification of genomic DNA as described by Wragg et al. [27]. The 25-µl amplification product was digested with 10 U restriction enzyme *Bam*HI at 37 °C for at least 3 h. Restriction fragments were separated by electrophoresis on a 3% NuSieve-1% agarose gel and visualized by ethidium bromide staining.

DNA diagnosis for HD was performed by determining the copy number for the CAG repeat in the IT-15 gene as described by Warner et al. [25], a method that determines the repeat length without including the polymorphic repeat slightly 3′ downstream to the (CAG)*n*.

Statistical analysis was performed using the χ^2 test, Student's *t* test, correlation analysis, and multivariate regression.

Results

Apolipoprotein E

The frequencies of *APOE* alleles in our series of patients and in controls are shown in Table 1. Allele ε4 had a frequency of 10.8% in our series not significantly different from 6.5% in the general population. The genotypes of the patients were ε2ε3:4, ε3ε3:44, ε3ε4:11, ε4ε4:1.

The average age at onset of our patients was 40.9 ± 11.1 years. The age at onset of the disease was significantly higher in the ε3ε4 patients (mean = 51.6 ± 8.7) than in ε3ε3 patients (mean = 38 ± 10.5; $t = 3.97$; $P < 0.0002$). To exclude possible interference of the number of repeats with our analysis we also performed multivariate regression analysis using the age at onset as a dependent variable and *APOE* genotype (ε3ε3 and ε3ε4) and CAG repeat length as independent variables. The analysis verified the known inverse correlation between CAG repeat length and age at onset (regression coefficient = –1.79; SE = 0.27; $t = -6.45$; $P < 0.001$), while it confirmed a strong independent correlation for *APOE* genotype, ε3ε4 individuals having a higher age at onset than those with ε3ε3 genotype (regression coefficient = 10.03; SE = 2.64; $t = 3.79$; $P < 0.001$).

Table 1 Distribution of *APOE* alleles in HD patients and controls

Subjects	Allele					
	ε2		ε3		ε4	
	n	%	*n*	%	*n*	%
HD patients	4	3.3	103	85.8	13	10.8
Control group	7	5.8	105	87.5	8	6.7
General population	23	5.3	381	88.2	28	6.5

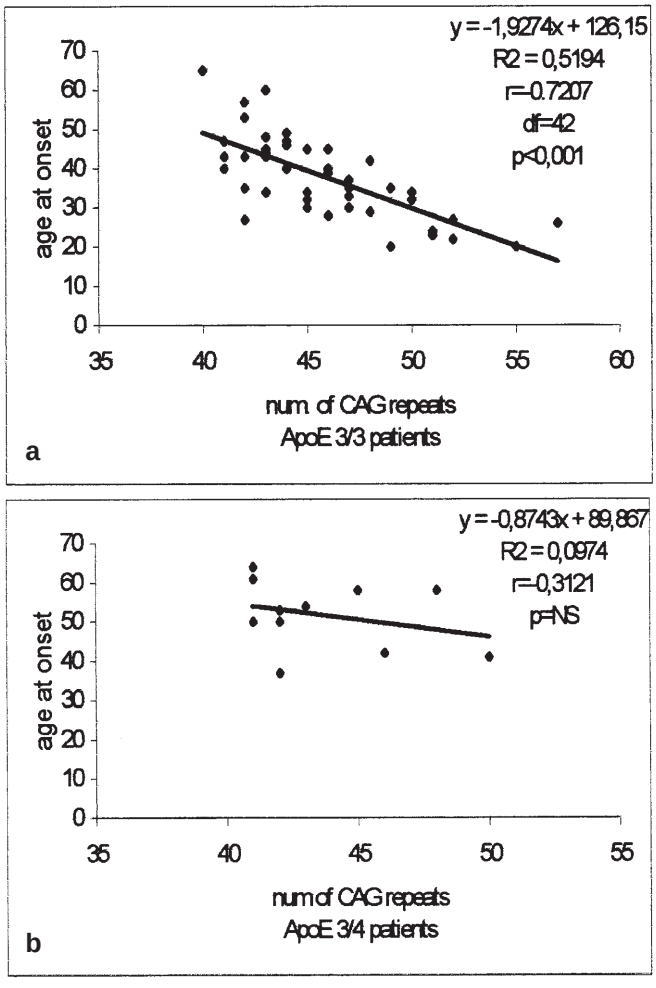

Fig. 1a, b Plots of age at onset against number of repeats for HD patients with the two major *APOE* genotypes. **a** ε3/ε3. **b** ε3/ε4. A strong correlation is observed in ε3/ε3 patients, stronger than observed in the overall series. The equation describing the correlation, the correlation coefficient and the value of *P* are noted. On the other hand, in the ε3/ε4 group no correlation was established

The correlation between age at onset and number of CAG repeats in the IT-15 was strong among patients with genotypes ε3ε3, but none was detected in patients with genotypes ε3ε4 (Fig. 1). No significant difference was found for the number of CAG repeats between *APOE*

ε3/ε3 and *APOE* ε3/ε4 group (mean = 45.7 ± 3.9 vs. 43.7±3.1; median = 45 vs. 42; range = 40–57 vs. 41–50; count = 44 vs. 11, respectively; $t = 1.58$; df = 53; $P = 0.119$). No significant differences in allele frequencies were detected between men and women either in patients or in controls.

Presenilin-1

No significant differences from our controls were detected concerning the allele frequencies (HD: allele 1 67%, allele 2 33%; control: allele 1 68%, allele 2 32%). Also no significant differences were found concerning the distribution of alleles in relation to age at onset or sex.

Discussion

The *APOE* genotype has been studied in a number of neurological disorders [5, 13, 15, 19, 22, 24], often with controversial results, and its role in the central nervous system remains unknown. In HD other investigators have previously studied the influence of *APOE* on the age at onset, but without positive findings [18]. Our analysis, using a different approach, revealed that patients carrying the *APOE* allele ε4 have a higher age at onset of HD than ε3ε3 patients ($P < 0.0002$), a finding remaining highly significant ($P < 0.001$) when multivariate regression was performed. It is of interest that although *APOE* has so far been regarded as an aging gene, whose ε4 allele has generally been viewed as the one responsible for accelerating age-related conditions, according to our data its presence is contributes to determining the age at onset of HD, in contrast to observations in other disorders.

When the patients with the two most common genotypes, ε3ε3 and ε3ε4, were used as a separate group to investigate the correlation of CAG repeat number with age at onset, the ε3/ε3 group presented a stronger correlation than that observed in all patients together while in the ε3/ε4 group no correlation could be established. The ε3/ε4 group consisted of only 11 individuals, which might account for the failure to detect the correlation. We believe, however, that this finding, although statistically not strongly supported, deserves further investigation.

To establish a model for determining the possible age at disease onset for those with expanded repeats in the IT-15 gene, our findings need to be further examined in larger groups and combined with data on other possible factors. The study of possible correlations between this and other candidate genes and the age at onset of HD could lead to a much more accurate prediction as well as a better understanding of the disease.

References

1. Andrew SE, Goldberg PY, Kremer B, et al (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington' s disease. Nat Genet 4:398–403
2. Avramopoulos D, Mikkelsen M, Vassilopoulos D, et al (1996) Apolipoprotein E allele distribution in parents of Down's syndrome children. Lancet 347:862–865
3. Chartier-Harlin M-C, Paofitt M, Legrain S, et al (1994) Apolipoprotein E, ε4 allele as a major risk factor for sporadic early and late-onset forms of Alzheimer's disease: analysis of the 19q13.2 chromosomal region. Hum Mol Genet 3:569–574
4. Corder EH, Saunders AM, Risch NJ, et al (1994) Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer's disease. Nat Genet 7:180–184
5. Couderc R, Mahieux F, Bailleul S, et al (1993) Prevalence of apolipoprotein E phenotypes in ischemic cerebrovascular disease. Stroke 24:661–664
6. Duijn CM van, de Knijff P, Wehnert A, et al (1995) The apolipoprotein E ε2 allele is associated with an increased risk of early-onset Alzheimer's disease and a reduced survival. Ann Neurol 37:605–610
7. Duyao M, Ambrose C, Myeus R, et al (1993) Trinucleotide repeat instability and age of onset in Huntington' s disease. Nat Genet 4:387–392
8. Farrer LA, Conneally PM, Ya P (1984) The natural history of Huntington' s disease: possible role of ageing genes. Am J Med Genet 18:115–123
9. Farrer LA, Conneally PM (1985) A genetic model for age at onset in Huntington' s disease. Am J Hum Genet 371:350–357
10. Finch CE (1980) The relationship of ageing changes in the basal ganglia to manifestation of Huntington' s chorea. Ann Neurol 7:406–410
11. Haldane JBS (1941) The relative importance of principal and modifying genes in determining some human diseases. J Genet 41:149–157
12. Harper PS (1996) Huntington's disease, 2nd edn. Sanders, London
13. Harrington CR, Louwagie J, Rossau R, et al (1994) Influence of apolipoprotein E genotype on senile dementia of the Alzheimer and Lewy body types. Am J Pathol 145:1472–1484
14. Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington' s disease chromosomes. Cell 72:971–983
15. Lippa CF, Smith TW, Saunders AM, et al (1995) Apolipoprotein E genotype and Lewy body disease. Neurology 45: 97–103
16. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:3–6
17. Rubinsztein DC, Leggo J, Coles R, et al (1996) Phenotypic Characterization of individuals with 30–40 CAG repeats in th Huntington disease (HD) gene reveals HD cases with 36 repeats and apparently normal elderly individuals with 36–39 repeats. Am J Hum Genet 59:16–22

18. Rubinsztein DC, Leggo J, Chiano M, 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 USA 94:3872–3876
19. Schneider J, Gearing M, Robins R, et al (1995) Apolipoprotein E genotype in diverse neurodegenerative disorders. Ann Neurol 38:131–135
20. Sklavounou E, Economou-Petersen E, Karadima G, et al (1997) Apolipoprotein E polymorphism in the Greek population. Clin Genet 52:216–218
21. Snell R, MacMillan J, Cheadle J, et al (1993) Relationship between trinucleotide repeat expantion and phenotypic variation in Huntington's disease. Nature Genet 4:393–397
22. Stina M, Rebeck W, McKenna-Yasek D, et al (1995) Apolipoprotein E ε4 allele is not associated with earlier age at onset in amyotrophic lateral sclerosis. Ann Neurol 38:460–463
23. Strittmatter WJ, Saunders AM, Schechel D, et al (1993) Apolipoprotein E: high-avidity binding to B-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 90: 1977–1981
24. Tabalon M, Rolleri M, Masturzo P, et al (1995) Apolipoprotein E ε4 allele frequency is not increased in progressive supranuclear palsy. Neurology 45: 1764–1765
25. Warner J, Barron L, Brook D (1993) A new polymerase chain reaction (PCR) assay for the trinucleotide repeat that is unstable and expanded on Huntington's disease chromosomes. Mol Cell Probes 7:235–239
26. Wenham PR, Price WH, Blundell G (1991) Apolipoprotein E genotyping by one-stage PCR. Lancet 337:1158–1159
27. Wragg M, Hutton M, Talbot C, the Alzheimer's Disease Collaborative Group (1996) Genetic association between intronic polymorphism in presenilin-1 gene and late onset Alzheimer's disease. Lancet 347:509–512