ELSEVIER

Neuroscience Letters 266 (1999) 149–151

Neuroscience Letters

Neurogenetic correlates of Parkinson's disease: apolipoprotein-E and cytochrome P450 2D6 genetic polymorphism

M.A.M. Bon[a], E.N.H. Jansen Steur[b], R.A.I. de Vos[c], I. Vermes[a,*]

[a]Department of Clinical Chemistry, Medical Spectrum Twente, Hospital Group, Enschede, P.O. Box 50000, NL-7500 KA, The Netherlands
[b]Department of Neurology, Medical Spectrum Twente, Hospital Group, Enschede, P.O. Box 50000, NL-7500 KA, The Netherlands
[c]Regional Laboratory for Pathology, Enschede, The Netherlands

Received 5 March 1999; accepted 15 March 1999

Abstract

Brain tissue of 50 patients with morphological confirmed Parkinson's disease (PD), blood samples from 149 patients with clinical parkinsonism and from 96 healthy control subjects were collected. Apolipoprotein-E (apo E) and cytochrome P450 2D6 (CYP2D6) genotyping were performed by PCR followed by restriction fragment analysis. A significantly higher allele frequency of CYP2D6*4 was found in patients with PD (35%) but not with parkinsonism (14.1%) compared to control subjects (19.8%). The combined alleles frequency of CYP2D6*3 + apoE4 was significantly higher not only in the PD group (33.3%) but also in patients with parkinsonism (22.3%) compared to control subjects (1.6%). These results suggest that there is a substantial overlap not only in the clinical manifestation but also in the genetic risk factors between Parkinson's and Alzheimer's diseases.

Keywords: Apolipoprotein-E; Cytochrome P450 2D6; Genetic polymorphism; Morphological confirmed Parkinson's disease; Progression of Parkinson's disease

Alzheimer's (AD) and Parkinson's diseases (PD) are both chronic neurodegenerative diseases characterized by a progressive decline in cognitive functions. Although AD and PD occur sporadically, both genetic and environmental factors acting either alone or in combination are believed to contribute to the initiation of these neurodegenerative disorders [5,8]. The interplay between genetically determined susceptibility traits and exposure to endogenous and exogenous neurotoxins stimulated the efforts to find genetic and environmental risk factors for these diseases [2]. Apolipoprotein-E (apoE), is a major apolipoprotein in the central nervous system, plays a key role in cholesterol transport and metabolism, is polymorphic and encoded by three alleles [11]. The presence of E4 alleles is a major risk for the development of AD, although the exact mechanism by which the presence of E4 alleles promotes this disease is not clear. The presence of E4 alleles is not causative but rather influence the age of onset of AD without effecting the progression of dementia [9].

However, genetic susceptibility which is associated with altered expression of enzymes regulating the metabolism of endogenous and exogenous neurotoxins is implicated in the pathomechanism of PD [10]. One of the most important factors in determining susceptibility to a xenobiotic agent is the metabolism. The absence of a metabolic pathway or a deficit in its function confers susceptibility to endogenous and exogenous toxins [3]. There is a vast number of enzyme systems including e.g. monoamine oxidase, superoxide dismutase, glutathione transferase, etc. which are involved in the metabolism of xenobiotics, but most attention has been focused on cytochrome P450 (CYP450) enzymes as susceptibility factors in PD [3,10]. Genetic polymorphism studies of CYP450 2D6 isoenzymes have shown that mutation of this enzyme is associated with a roughly doubled risk to acquire PD [3,10].

The diagnosis of both PD and AD is made on the basis of clinical criteria but the gold standard remains the neuropathological examination [8]. The definitive diagnosis can only be made after post-mortem examination, because a substantial part of the patients (24–40%) with clinical diagnosis 'Parkinsonism' do not have PD [6,8]. The low accuracy of the clinical diagnosis is due to the fact that there is a substantial overlap between the clinical symptoms of these two diseases. Dementia is an important feature of PD on one hand, and parkinsonian signs are common in AD on the

* Corresponding author. Tel.: +31-53-487-3404; fax: +31-53-487-3075.
E-mail address: i.vermes@wxs.nl (I. Vermes)

0304-3940/99/$ - see front matter
PII: S0304-3940(99)00278-5

Table 1
The allele frequency and combined frequency of CYP2D6 mutations and apoE subtypes in three different groups of subjects[a]

	PD ($n = 50$)			Parkinsonism ($n = 149$)			Control (n = 96)
	%	$P < 0.05$	OR	%	$P < 0.05$	OR	%
2D6*4	35	<	2.2	14.1	>	0.6	19.8
2D6*3	6	>	2.0	3.7	>	1.3	3.0
APO–E2	12	>	1.9	10.1	>	1.5	6.8
APO–E3	69	<	0.5	73.1	>	0.7	79.7
APO–E4	19	>	1.6	16.7	>	1.4	12.5
	Combined allele frequence						
*4 + E2	14.1	<	2.4	7.0	>	1.1	6.0
*4 + E3	68.7	<	0.6	75.0	>	0.8	78.8
*4 + E4	17.2	>	1.1	18.0	>	1.2	15.1
*3 + E2	0		0				6.7
*3 + E3	66.7	<	0.2	77.7	<	0.3	91.7
*3 + E4	33.3	<	23	22.3	<	13	1.6

[a] Parkinson's disease (PD) versus control, parkinsonism versus control; OR, odds ratio.

other hand. Almost 50% of PD patients have dementia in life, but half of these patients in postmortem investigation do have coexisting AD [8]. The question is whether this overlap is due to clinical misdiagnosis and diagnostic pitfalls or due to the same genetic susceptibility. In order to address this question we studied the presence of a genetic risico factor typical for AD, apoE in PD patients. The effect of the clinical misdiagnosis was studied by comparing the combined allele frequencies of CYP2D6*3, CYP2D6*4 and apoE-subtypes in patients with neuropathologically confirmed PD (PD), in patients with only clinical diagnosis of PD (parkinsonism) and in healthy control subjects.

Brain tissue speciments were obtained from 50 PD patients, and blood samples were collected from 149 patients with parkinsonism and from 96 healthy blood bank donors (control group). The procedures followed are in accordance with the policies of the institutional ethical review board of the Hospital Group (ECOM) according to the Declaration of Helsinki 1957, as revised in 1983. The neuropathological diagnostic criteria for PD were the presence of Lewy bodies and Lewy neurites. The quantitative analysis of Lewy bodies, neuritic plaques and neurofibrillary tangles were performed as described previously [13]. Eighteen morphologicaly confirmed PD patients had coexisting AD (36%), and 35 of the PD patients (70%) were demented (mini mental sate examination, MMSE, score < 21). The clinical diagnosis of parkinsonism was made when minimal two of the following symtoms were present: tremor, rigidity, bradykinesia, postural disturbances [8].

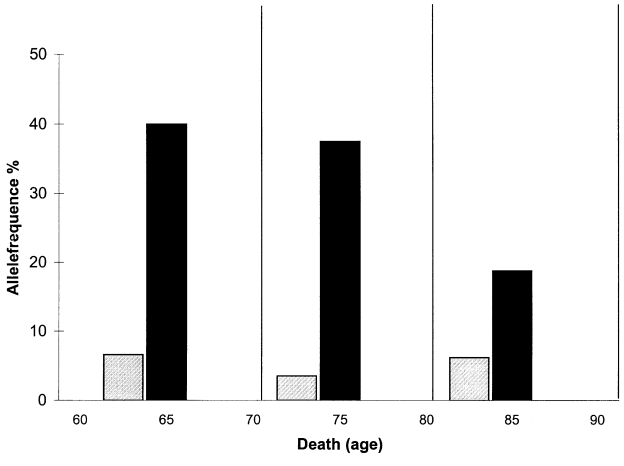

Fig. 1. Allele frequencies CYP2D6*3 (hatched box) and CYP2D6*4 (black box) according to the age of patients with morphological confirmed Parkinson's disease.

DNA from formalin fixed paraffin-embedded brain material and from blood samples was isolated according to our previously published method [1]. Apolipoprotein-E genotyping was performed by PCR (for brain tissue followed by semi-nested PCR). Afterwards the subtypes E2, E3 and E4 were determined with restriction fragment analysis [7]. The CYP2D6 genotyping was also performed by PCR, primer sequences CYP2D6*3, forward 5′- GAT GAG CTG CTA ACT GAG CCC -3′, reverse 5′- CCG AGA GCA TAC TCG GGA C -3'. Primer sequences CYP2D6*4, forward 5'- GCC TTC GCC AAC CAC TCC G -3′, reverse 5′- AAA TCC TGC TCT TCC GAG GC -3′ (brain tissue underwent reamplification with the same primers), digestion and gel electrophoresis [4,12]. Statistical analysis was performed by using the χ^2-test at $P < 0.05$.

In accordance with the literature [3,10] we found a significantly higher mutant allele frequency of CYP2D6*4 in PD patients resulting a significantly higher odds ratio of 2.2 ($P < 0.05$) (Table 1). In addition, the mutant CYP2D6*4 allelle was associated with a rapid progression of PD: the group of PD patients who died within 10 years after the onset of the disease had a significantly higher allele frequency than the group with a disease duration of more than 10 years (Fig. 1). However these frequencies were found only in the PD group and not in the patient group

with parkinsonism, suggesting a significant amount of misdiagnosis in this group. The mutant allele frequency of CYP2D6*3 in PD patients (6%) was increased compared to the parkinsonism patients (3.7%) and to the control subjects (3%) (odds ratio of 2.0, Table 1).

The most striking result of our study appeared when CYP2D6*3 was combined with apoE4. We observed this combined allele frequency CYP2D6*3 and E4 in the PD group in 33.3%, in the parkinsonism group 22.3%, and in the control group in 1.6% (Table 1). The odds ratios were highly significant for both groups: 23.0 and 13.0 for the PD and for the parkinsonism groups, respectively, compared with the control group. In addition, the combined allele frequency of CYP2D6*4 plus apoE2 proved to be also significantly higher in the PD group (14.1%) compared to the control group (6.0%) resulting a significantly higher odds ratio for PD (2.4, $P < 0.05$).

There is increasing evidence that PD may have more than one cause. The results of our study are suggesting at least two genetic risks: CYP2D6 and apoE polymorphism, and show that the association of these two factors can increase significantly PD risk. In accordance with recent literature we found only a modest increases in PD risk associated with the mutant allele CYP2D6*4. However, we demonstrated that CYP2D6*4 is associated with a rapid progression of PD resulting a significantly lower survival in the mutant group. In the same group we found a higher frequency of apoE2 allele but not apoE4 allele, suggesting that the lower survival is not due to natural selection. Based on recent literature one could expect a higher survival and a lower AD rate in the presence of apoE2 allele [11]. There is increasing evidence that the apoE2 allele is associated with longevity and protects against AD. Supporting this view, we were unable to demonstrate the same effect in the group of parkinsonism patients, suggesting a higher rate of clinical misdiagnosis in this group.

However, an overlap in the genetic susceptibility of PD and AD has been demonstrated when we found a highly significant increase in odds ratios for PD in the presence of CYP2D6*3 and apoE4 alleles. Our data suggest that the high prevalence of AD among PD patients and vice versa not only due to clinical misdiagnosis but might genetically predisposed. Apolipoprotein-E4 appears to play a central role in the development of several neurodegenerative processes, but according to the general view plays no role in the pathogenesis of PD. We and others [1,11] did not find increased apoE4 frequency in PD. According to our knowledge, this is the first study to demonstrate a significantly increased combined alleles frequency of apoE4 and CYP2D6 in patients with PD. Based on this observation we can speculate that there is a significant overlap not only in the clinical symptoms but also in the genetical susceptibility between PD and AD.

[1] Ballering, L.A.P., Steffens-Nakken, H.M., Esselink, R.A.J., de Vos, R.A.I., Jansen Steur, E.N.H. and Vermes, I., Apolipoprotein E genotyping in patients with neurogenerative diseases. Clin. Biochem., 30 (1997) 405–411.
[2] Bandmann, O., Marsden, C.D. and Wood, N.W., Genetic aspects of Parkinson's disease. Mov. Disord., 13 (1998) 203–211.
[3] Checkoway, H., Farin, F.M., Costa-Mallen, P., Kirchner, S.C. and Costa, L.G., Genetic polymorphism in Parkinson's disease. NeuroToxicology, 19 (1998) 635–644.
[4] Douglas, A.M., Atchison, B.A., Somogyi, A.A. and Drummer, O.H., Interpretation of a simple PCR analysis of the CYP2D6(A) and CYP2D6(B) null alleles associated with the debrisoquine/sparteine genetic polymorphism. Pharmacogenetics, 4 (1994) 154–158.
[5] Hardy, J. and Gwinn-Hardy, K., Genetic classification of primary neurodegenerative disease. Science, 282 (1998) 1075–1079.
[6] Hughes, A.J., Daniel, S.E., Kilford, L. and Lees, A.J., Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J. Neurol. Neurosurg. Psychiatry, 55 (1992) 181–184.
[7] Kontula, K., Setälä, K.A., Kuusi, T., Hämäläinen, L. and Syvänen, A.C., Apolipoprotine E polymorphism determined by restriction enzyme analysis of DNA amplified by PCR. Clin. Chem., 36 (1990) 2087–2092.
[8] Lang, A.E. and Lozano, A.M., Parkinson's disease, first of two parts. N. Eng. J. Med., 339 (1998) 1044–1053.
[9] Price, D.L., Sisodia, S.S. and Borchelt, D.R., Genetic neurodegenerative diseases: the human illness and transgenic models. Science, 282 (1998) 1079–1083.
[10] Riedl, A.G., Watts, P.M., Jenner, P. and Marsden, C.D., P450 Enzymes and Parkinson's disease: the story so far. Mov. Disord., 13 (1998) 212–220.
[11] Siest, G., Pillot, T., Regis-Bailly, A., Leininger-Muller, B., Steinmetz, J., Galteau, M.M. and Visvikis, S., Apolipoprotein E: important gene and protein to follow in laboratory medicine. Clin. Chem., 41 (1995) 1068–1086.
[12] Steijns, S.W. and Weide van der, J., Ultrarapid drug metabolism: PCR-based detection of CYP2D6 gene duplication. Clin. Chem., 44 (5) (1998) 1–4.
[13] Vos de, R.A.I., Jansen, E.N.H., Stam, F.C., David, R. and Swaab, D.F., 'Lewy body disease': clinicopathological correlations in 18 consecutive cases of Parkinson's disease with and without dementia. Clin. Neurol. Neurosurg., 97 (1995) 13–22.