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Dipeptidyl carboxypeptidase 1 (*DCP1*) and butyrylcholinesterase (*BCHE*) gene interactions with the apolipoprotein E ε4 allele as risk factors in Alzheimer's disease and in Parkinson's disease with coexisting Alzheimer pathology

Kari M Mattila, Juha O Rinne, Matias Röyttä, Pekka Laippala, Timo Pietilä, Hannu Kalimo, Timo Koivula, Harry Frey, Terho Lehtimäki

Department of Clinical Chemistry, Centre for Laboratory Medicine, Tampere University Hospital, Finn-Medi 2, Third Floor, PO Box 2000, FIN-33521 Tampere, Finland
K M Mattila
T Koivula
T Lehtimäki

Department of Neurology, University of Turku, Turku, Finland
J O Rinne

Department of Pathology, University of Turku, Turku, Finland
M Röyttä
H Kalimo

Research Unit, Tampere University Hospital, Tampere, Finland
P Laippala

School of Public Health, Medical School, University of Tampere, Tampere, Finland
P Laippala

Department of Neurology, Tampere University Hospital, Tampere, Finland
T Pietilä
H Frey

Department of Neurology, Medical School, University of Tampere, Tampere, Finland
H Frey

Correspondence to:
Dr Mattila, klkama@uta.fi

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Abstract

Alzheimer's disease (AD) and Parkinson's disease (PD) are genetically heterogeneous. Dipeptidyl carboxypeptidase 1 (*DCP1*) and butyrylcholinesterase (*BCHE*) genes may modify the risk of these disorders. We investigated whether common polymorphisms present in these genes operate as risk factors for AD and PD in Finnish subjects, independently or in concert with the apolipoprotein E ε4 allele (*APOE* ε4). Eighty late onset sporadic AD patients, 53 PD patients (34 of whom had concomitant AD pathology), and 67 control subjects were genotyped for the insertion (I)/deletion (D) polymorphism of *DCP1* and the K variant of *BCHE*. In logistic regression analysis, the *DCP1* *I allele in combination with *APOE* ε4 significantly increased the risk of AD (OR 30.0, 95% CI 7.3-123.7), compared to subjects carrying neither of the alleles. Similar analysis showed that the risk of AD was significantly increased in subjects carrying both the *BCHE* wild type (*WT/*WT) genotype and ε4 (OR 9.9, 95% CI 2.9-33.8), compared to those without this *BCHE* genotype and ε4. Further, the risk of PD with AD pathology was significantly increased for carriers of *DCP1* *I and ε4 (OR 8.0, 95% CI 2.1-31.1). We thus conclude that, in Finns, interaction between *DCP1* *I and ε4 increases the risk of AD as well as of PD with coexisting Alzheimer pathology, which underlines the importance of the *DCP1* I/D polymorphism in the development of Alzheimer neuropathology, whereas the wild type *BCHE* genotype in combination with ε4 had a combined effect with regard to the risk of AD.

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Keywords: Alzheimer's disease; Parkinson's disease; dipeptidyl carboxypeptidase 1; butyrylcholinesterase

Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most frequent neurodegenerative disorders with heterogeneous aetiologies including genetic factors. Mutations in the amyloid precursor protein and the presenilin genes are known to cause familial forms of AD,[1] whereas the λ-synuclein and parkin genes, when mutated, are responsible for rare familial cases of PD.[2] Furthermore, the ε4 allele of apolipoprotein E (*APOE* ε4) has been identified as the most significant risk factor for late onset familial and sporadic AD.[3] In PD, the frequency of *APOE* ε4 has been found to be increased in cases with concomitant AD pathology.[4] Since a significant proportion of sporadic AD patients have no ε4, other genetic factors must play a role in the pathogenesis of the disorder. It has also become evident that genetic polymorphisms may contribute to the development of PD.[5 6] Genes encoding dipeptidyl carboxypeptidase 1 (DCP1) (also known as angiotensin I converting enzyme (ACE)) and butyrylcholinesterase (BCHE) may modify the risk of these disorders. The deletion (D) allele of the insertion/deletion polymorphism of the *DCP1* gene has been reported to be associated with longevity,[7] whereas the insertion (I) allele has recently been found to confer susceptibility to AD,[8] while neither allele has so far been observed to be associated with PD.[9] The K variant of the *BCHE* gene has been implicated in both AD[10] and PD.[11] Verification of the contribution of these two genes to AD and PD requires their examination for association with these disorders in different populations. Our purpose here was to establish whether the *DCP1* I/D and *BCHE* WT/K polymorphisms operate as risk factors for AD and PD in Finnish subjects, independently or in conjunction with *APOE* ε4.

Materials and methods

Approval for the study was obtained from the local Hospital Ethical Committees. All patients and control subjects enrolled in the investigation were from the western part of Finland. The AD group consisted of 80 late onset (>65 years) sporadic patients (51 women, 29 men). Twenty seven patients (mean age at onset 73.5 years (SD 6.0)) had a clinical diagnosis of probable AD made according to the NINCDS-ADRDA criteria,[12] while for 53 cases (mean age at death 80.4 years (SD 5.5)) the clinical diagnosis had been confirmed neuropathologically using the CERAD criteria.[13] The PD group comprised 53 neuropathologically verified patients (27 women, 26 men; mean age at death 75.9 years (SD 6.3)). The pathological diagnosis of PD was based on the loss of

Table 1 Genotype and allele distributions of the DCP1 I/D polymorphism in the AD, PD, PD(B+C), and control groups

Group		Genotypes			Alleles	
		II	ID	DD	I	D
AD	n=80	16 (0.20)	49 (0.61)	15 (0.19)*	81 (0.51)	79 (0.49)
PD	n=53	12 (0.23)	27 (0.51)	14 (0.26)	51 (0.48)	55 (0.52)
PD(B+C)	n=34	9 (0.26)	19 (0.56)	6 (0.18)	37 (0.54)	31 (0.46)
Controls	n=67	10 (0.15)	31 (0.46)	26 (0.39)	51 (0.38)	83 (0.62)
ε4 carriers						
AD	n=57	12 (0.21)	33 (0.58)	12 (0.21)	57 (0.50)	57 (0.50)
PD	n=23	6 (0.26)	12 (0.52)	5 (0.22)	24 (0.52)	22 (0.48)
PD (B+C)	n=18	6 (0.33)	10 (0.56)	2 (0.11)	22 (0.61)	14 (0.39)
Controls	n=17	1 (0.06)	8 (0.47)	8 (0.47)	10 (0.29)	24 (0.71)
ε4 non-carriers						
AD	n=23	4 (0.17)	16 (0.70)	3 (0.13)	24 (0.52)	22 (0.48)
PD	n=30	6 (0.20)	15 (0.50)	9 (0.30)	27 (0.45)	33 (0.55)
PD (B+C)	n=16	3 (0.19)	9 (0.56)	4 (0.25)	15 (0.47)	17 (0.53)
Controls	n=50	9 (0.18)	23 (0.46)	18 (0.36)	41 (0.41)	59 (0.59)

*AD *v* controls (II+ID/DD), p=0.007.
II, *DCP1* *I/*I; ID, *DCP1* *I/*D; DD, *DCP1* *D/*D; I, *DCP1* *I; D, *DCP1* *D
n, number of subjects genotyped; frequencies shown in parentheses.

pigmented neurones in the substantia nigra with gliosis, pigment phagocytosis, and Lewy bodies. The PD cases were divided into different groups according to the CERAD criteria.[13] Thirty four PD cases yielded histological findings suggestive (group B) or indicative (group C) of AD (referred to as the PD(B+C) group), whereas 13 showed no histological evidence of AD (group 0). In the remaining six PD cases, the evidence for AD was uncertain (group A, n=3) or no information was available on possible concomitant AD pathology (n=3). The 67 controls were elderly subjects with no clinical signs of neurological or psychiatric disease (30 women, 37 men; mean age 75.9 years (SD 7.3)). In 59 cases, brain tissue had been obtained at necropsy and verified to be normal based on a thorough neuropathological investigation made according to CERAD recommendations.[13] Amygdala, hippocampus (CA 1 sector), substantia nigra, and five cortical gyri (medial frontal, rectus, cingulate, angular, medial temporal) were examined for senile plaques, neurofibrillary tangles, and Lewy bodies as well as for other possible neuropathology. Additional samples from striatum, pons, medulla oblongata, and cerebellum were also taken for neuropathological examinations.

Genomic DNA was extracted from whole blood or postmortem brain tissue by standard methods (QIAamp kits, Qiagen, USA). The I/D polymorphism of the *DCP1* gene was detected using the protocol of Odawara *et al*,[14] where possible mistyping of the *DCP1* *I/*D genotype as *D/*D was controlled by means of insertion specific primers and the inclusion of dimethyl sulphoxide in the PCR reaction. The amplification created restriction site method of Jensen *et al*[15] was used to identify the K variant of the *BCHE* gene. *APOE* ε4 allele status was determined as described elsewhere.[16]

The test for Hardy-Weinberg equilibrium was carried out using Arlequin (version 1.1, Schneider S, Kueffer JM, Roessli D, and Excoffier L, Genetics and Biometry Laboratory, University of Geneva, Switzerland, 1997). The genotype and allele frequencies of the *DCP1* and *BCHE* genes were compared between cases and controls using the chi-square test. Multinomial logistic regression analysis (SPSS 9.0 for Windows, SPSS Inc, 1999) was used to investigate the risk of AD or PD produced by *APOE* ε4 (one or two alleles) and *DCP1* *I or *DCP1* *D. A custom model was created to estimate interaction between ε4 and *DCP1* *I or *DCP1* *D, taking as reference subjects who possessed neither ε4 nor *DCP1* *I (ε4−/*DCP1* *I−) or *DCP1* *D (ε4−/*DCP1* *D−). The risk of AD or PD produced by ε4 status/*BCHE* *WT or ε4 status/*BCHE* *K combinations was analysed using the same approach. In view of the simultaneous comparisons, the correction factor n(m-1) (n loci with m alleles each) was applied to correct the significance level ($p<0.008$).

Results

The distributions of genotypes and alleles of the *DCP1* I/D polymorphism in the AD, PD, PD(B+C), and control groups are presented in table 1. All groups were in Hardy-Weinberg equilibrium. The frequency of the *DCP1* *I/*I and *DCP1* *I/*D genotypes (*DCP1* *I carriers) compared to the *DCP1* *D/*D genotype (*DCP1* *I non-carriers) was significantly higher in the AD group compared to controls (p=0.007). In the PD and PD(B+C) groups the frequencies of the genotypes and alleles of *DCP1* I/D did not differ significantly from those observed in the control group.

Calculation of an odds ratio (OR) for carriers of *APOE* ε4, for carriers of *DCP1* *I or *DCP1* *D, and for those possessing ε4 in combination with *DCP1* *1 or *DCP1* *D (subjects with neither ε4 nor *DCP1* *I or *DCP1* *D as reference) (table 2), showed that the risk of AD was significantly increased for those who carried both *DCP1* *I and ε4 (OR 30.0, 95% confidence interval (CI) 7.3-123.7). The overall risk was greater than with either of these

*Table 2 Risk of AD and PD for carriers of APOE ε4, DCP1 *I, or DCP1 *D, or both alleles (ε4 in combination with DCP1 *I or DCP1 *D), using subjects having neither ε4 nor DCP1 *I or DCP1 *D as reference*

	AD		PD		PD (B+C)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
ε4+/*DCP1* *I+	30.0 (7.3–123.7)	<0.0001	4.0 (1.3–12.4)	0.016	8.0 (2.1–31.1)	0.003
ε4+/*DCP1* *I−	9.0 (2.0–40.9)	0.004	1.3 (0.3–4.9)	0.750	1.1 (0.2–7.5)	0.903
ε4−/*DCP1* *I+	3.8 (1.0–14.4)	0.054	1.3 (0.5–3.5)	0.583	1.7 (0.5–6.0)	0.420
ε4−/*DCP1* *I−	Ref		Ref		Ref	
ε4+/*DCP1* *D+	6.3 (1.7–23.4)	0.006	1.6 (0.5–5.5)	0.461	2.3 (0.5–10.1)	0.291
ε4+/*DCP1* *D−	27.0 (2.6–284.7)	0.006	9.0 (0.9–94.9)	0.068	18.0 (1.5–216.6)	0.023
ε4−/*DCP1* *D+	1.0 (0.3–3.8)	0.950	0.9 (0.3–2.8)	0.824	1.0 (0.2–4.0)	0.946
ε4−/*DCP1* *D−	Ref		Ref		Ref	

OR, odds ratio; CI, confidence interval.

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Table 3 Genotype and allele distributions of the BCHE WT/K polymorphism in the AD, PD, PD(B+C), and control groups

Group		Genotypes			Alleles	
		WW	WK	KK	W	K
AD	n=80	60 (0.75)	17 (0.21)	3 (0.04)	137 (0.86)	23 (0.14)
PD	n=53	39 (0.73)	12 (0.23)	2 (0.04)	90 (0.85)	16 (0.15)
PD(B+C)	n=34	22 (0.65)	10 (0.29)	2 (0.06)	54 (0.79)	14 (0.21)
Controls	n=67	48 (0.72)	18 (0.27)	1 (0.01)	114 (0.85)	20 (0.15)
ε4 carriers						
AD	n=57	42 (0.74)	12 (0.21)	3 (0.05)	96 (0.84)	18 (0.16)
PD	n=23	14 (0.61)	7 (0.30)	2 (0.09)	35 (0.76)	11 (0.24)
PD(B+C)	n=18	10 (0.56)	6 (0.33)	2 (0.11)	26 (0.72)	10 (0.28)
Controls	n=17	11 (0.65)	5 (0.29)	1 (0.06)	27 (0.79)	7 (0.21)
ε4 non-carriers						
AD	n=23	18 (0.78)	5 (0.22)	0 (0.00)	41 (0.89)	5 (0.11)
PD	n=30	25 (0.83)	5 (0.17)	0 (0.00)	55 (0.92)	5 (0.08)
PD(B+C)	n=16	12 (0.75)	4 (0.25)	0 (0.00)	28 (0.88)	4 (0.12)
Controls	n=50	37 (0.74)	13 (0.26)	0 (0.00)	87 (0.87)	13 (0.13)

WW, *BCHE* *WT/*WT; WK, *BCHE* *WT/*K; KK, *BCHE* *K/*K; W, *BCHE* *WT; K, *BCHE* *K
n, number of subjects genotyped; frequencies shown in parentheses.

alleles alone. The high OR obtained for the ε4+/*DCP1* *D− combination (that is, ε4 carriers with the *DCP1* *I/*I genotype) when compared with ε4−/*DCP1* *D− (that is, ε4 non-carriers with the *DCP1* *I/*I genotype) could be explained by interaction between *DCP1* *I and *APOE* ε4. The risk of PD was similarly increased for those who carried *DCP1* *I in combination with ε4, and it was doubled when PD(B+C) cases were analysed separately (OR 8.0, 95% CI 2.1-31.1) (table 2). No increased risk of PD without AD pathology could be observed for ε4 status/*DCP1* *I combinations (data not shown).

The distribution of genotypes of the *BCHE* WT/K polymorphism did not diverge significantly from Hardy-Weinberg equilibrium in any of the patient or control groups studied. The genotype and allele frequencies of this polymorphism in AD, PD, and PD(B+C) patients did not differ significantly from those found in controls (table 3). No increased risk of AD, PD, or PD with AD pathology resulting from interaction between ε4 and *BCHE* *K could be observed, taking as reference subjects who carried neither of the alleles (table 4). Since all ε4 non-carriers had at least one *BCHE* *WT allele, the risk of AD or PD for subjects possessing *BCHE* *WT in combination with ε4 could not be estimated. In contrast, investigation of the influence of the *BCHE* *WT/*WT genotype and *APOE* ε4 allele status on the risk of AD showed a significantly increased risk of the disease in patients with both the *BCHE* *WT/*WT genotype and ε4, taking as reference subjects who had neither this *BCHE* genotype nor ε4. The OR for developing AD was calculated as 9.9 (95% CI 2.9-33.8, p<0.0001). *APOE* ε4 and the *BCHE* *WT/*WT genotype alone gave an OR of 6.5 (95% CI 1.6-26.4, p=0.009) and 1.3 (95% CI 0.4-4.1, p=0.695), respectively. In the PD and PD(B+C) groups, no significantly increased risk was observed for the ε4+/*BCHE* *WT/*WT+ combination (data not shown).

Discussion

In the present study, we found evidence that interaction between *DCP1* *I and ε4 significantly increases the risk of AD compared to the situation in subjects without the two alleles. In PD, interaction between *DCP1* *I and ε4 could also be observed, but the association with the disorder was restricted to PD cases with histological findings suggestive or indicative of AD, which lends further support to the view that the *DCP1* I/D polymorphism may be of importance in the development of AD neuropathology.

Our finding that the *DCP1* *I carrier frequency was significantly increased in the Finnish late onset sporadic AD group compared to controls is in agreement with that of Kehoe *et al*,[8] who reported a similar association between *DCP1* I/D and AD in the British population. Alvarez *et al*[17] found the increased *DCP1* *I carrier frequency of a Spanish AD group not to differ significantly from that in controls, but saw a significantly increased frequency of the *DCP1* *I allele in AD patients over controls. In contrast, no association between *DCP1* I/D and AD was observed in Israeli[18] or Italian[19] subjects, whereas in another Italian sample the *DCP1* *D allele was found to be implicated in this disorder.[20] In the present investigation, the genotyping of the *DCP1* I/D polymorphism was carried out using the method of Odawara *et al*,[14] the most accurate PCR based technique for the purpose. In some studies, identification of the heterozygous *DCP1* *I/*D genotype as the homozygous *D/*D genotype may have been a source of inaccurate results, since such mistyping has been found to occur frequently when earlier modifications of *DCP1* I/D genotyping were used.[14]

The mechanism whereby the *DCP1* I/D polymorphism could contribute to the development of AD pathology remains obscure. Since the polymorphism is located within an intron, it is conceivable that the *DCP1* *I allele is not a genetic factor predisposing to AD, but exists in linkage disequilibrium with a variant elsewhere in the *DCP1* gene or with a locus nearby. Further, if the finding that *DCP1* *D constitutes a risk factor for AD in some populations is confirmed, this again suggests that the *DCP1* I/D polymorphism is not a susceptibility factor in the disease.

*Table 4 Risk of AD and PD for carriers of APOE ε4, BCHE *K, or both alleles, using subjects having neither of the alleles as reference*

	AD		PD		PD(B+C)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
ε4+/*BCHE* *K+	5.1 (1.7–15.5)	0.004	2.2 (0.7–7.0)	0.174	4.1 (1.2–14.2)	0.026
ε4+/*BCHE* *K−	7.8 (3.3–18.7)	<0.0001	1.9 (0.7–4.8)	0.186	2.8 (1.0–8.2)	0.060
ε4−/*BCHE* *K+	0.8 (0.2–2.6)	0.695	0.6 (0.2–1.8)	0.337	1.0 (0.3–3.5)	0.937
ε4−/*BCHE* *K−	Ref		Ref		Ref	

OR, odds ratio; CI, confidence interval.

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The role of the WT/K polymorphism of *BCHE* in the development of AD is unclear. The initial report by Lehmann *et al*,[10] where white British subjects were studied, suggested that the K variant of the *BCHE* gene and *APOE* ε4 interact, increasing the risk of late onset AD. Their finding has been replicated in investigations in western Australian patients,[21] white Canadians,[22] and again in white British subjects,[23] while studies on patients from eastern Finland[24] and white Australians[25] have found evidence that the K variant could have a protective effect against AD. The majority of investigations[26–35] so far have, however, failed to establish an association between *BCHE* WT/K and AD. In the present study, subjects possessing the *BCHE* *WT/*WT genotype in combination with *APOE* ε4 were observed to run an increased risk of the disease compared to those having neither this *BCHE* genotype nor ε4. Considering the role BCHE might have in the development of AD, for example, in the maturation process of senile plaques,[36 37] and the fact that *BCHE**K produces a marked reduction in BCHE enzyme activity, the *BCHE* *WT allele, but not the K variant, could be expected to be implicated in AD. According to Singleton *et al*,[11] *BCHE* WT/K may also have a role in the development of PD, but we found no evidence to support this.

Lehmann *et al*[10] reported that the effect of the K variant on the risk of AD was seen more clearly in a subgroup of patients with an age at disease onset exceeding 75 years. One explanation for the contradictory results obtained in studies on the role of the *BCHE* WT/K polymorphism in AD could therefore be failure to analyse a specific and narrower onset age stratum, leaving some of the effects of the polymorphism on the risk of AD undetected (as, for example, in the present study owing to unavailability of the exact age at onset for all AD cases). The *BCHE* WT/K polymorphism may also exist in linkage disequilibrium with another nearby locus which is the actual susceptibility factor. Differences in the strength of this linkage disequilibrium in different populations could explain conflicting findings regarding the role of the K variant in AD. The data of Brindle *et al*[26] did not, however, support this hypothesis.

In conclusion, our data showed that in Finns interaction between *DCP1* *I and ε4 increased the risk of AD as well as that of PD with coexisting AD pathology, whereas the wild type *BCHE* genotype in combination with ε4 increased the risk of AD. The possibility exists, however, that the variants of the *DCP1* I/D and *BCHE* WT/K polymorphisms identified here as being associated with AD and PD with concomitant AD pathology are in fact not susceptibility factors but are in linkage disequilibrium with such factors present elsewhere in these genes or in loci in their proximity.

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