RESEARCH PAPER

Prion 6:1, 73-80; January/February/March 2012; © 2012 Landes Bioscience

PRND 3'UTR polymorphism may be associated with behavioral disturbances in Alzheimer disease

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Key words: Alzheimer disease, mild cognitive impairment, behavioral symptoms, *APOE*, *CYP46*, *PRNP*, *PRND*, polymorphisms

Abbreviations: Aβ, beta-amyloid; AD, Alzheimer disease; BPSD, behavioral and psychological symptoms of dementia; CDR, Clinical Dementia Rating scale; CJD, Creutzfeldt-Jakob disease; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory; SNP, single-nucleotide polymorphism; UTR, untranslated region

The etiology of behavioral and psychological symptoms of dementia (BPSD) is complex, including putative biological, psychological, social and environmental factors. Recent years have witnessed accumulation of data on the association between genetic factors and behavioral abnormalities in Alzheimer disease (AD). In this research paper, our aim was to evaluate the association between the *APOE*, *CYP46*, *PRNP* and *PRND* genes and the profile of neuropsychiatric symptoms in Polish subjects with AD and mild cognitive impairment (MCI). We studied 99 patients with AD and 48 subjects with MCI. The presence and profile of BPSD were evaluated at baseline and prospectively with the Neuropsychiatric Inventory (NPI). Patients were dichotomized into those having ever experienced a particular symptom and those who did not over the whole disease period. Genotyping was performed using previously described standard protocols. The prevalence of comorbid behavioral symptoms and the overall level of behavioral burden were significantly greater in AD compared with the MCI group. In AD patients, carrier status of the T allele of the 3'UTR (untranslated region) *PRND* polymorphism was associated with an increased cumulative behavioral load and an elevated risk for delusions, anxiety, agitation/aggression, apathy and irritability/emotional ability. Among MCI subjects, *APOE* ε4 carriers demonstrated a reduced risk for nighttime behavior change. No other statistically significant genotype-phenotype correlations were observed, including the *APOE*, *CYP46* and *PRNP* genes. A precise estimation of the exact significance of particular polymorphisms in BPSD etiology requires future studies on large populations.

Introduction

Alzheimer disease (AD) is the most common cause of dementia, accounting for approximately 70% of cases in subjects over 70 years.[1] Cognitive impairment leading to functional decline, dementia defining symptom, is frequently accompanied by diverse behavioral changes and neuropsychiatric symptoms clustered together as BPSD (behavioral and psychological symptoms of dementia). In the course of AD, BPSD are present in nearly all patients, with an average of around 90%,[2] although their prevalence and profile changes with dementia severity. Moreover, BPSD are a common phenomenon across all stages of cognitive decline—even in mild cognitive impairment (MCI), often considered a prodromal phase of AD, 60% of subjects suffered from at least one neuropsychiatric symptom.[3] The clinical significance of BPSD corresponds to the more aggressive disease course, including more rapid cognitive and functional decline, elevated mortality, early institutionalization and substantially increased caregiver burden.[4] In the context of the clinical, economic and social consequences of BPSD, discovering mechanisms implicated in their pathogenesis is among the top-priority challenges of old-age psychiatry.

The etiology of BPSD is complex, including putative biological, psychological, social and environmental factors. Recent years have witnessed accumulation of data on the association between genetic factors and behavioral abnormalities in AD. Multiple genes have been assessed for their putative association with BPSD risk. The most extensively studied comprise *APOE*, encoding for apolipoprotein E (apoE) and genes encoding for proteins involved in the process of neurotransmission: serotonin receptors, serotonin transporter, catechol-O-methyltransferase, dopamine receptors, monoamine oxidase A or tryptophane hydroxylase (reviewed in refs. 5 and 6). The involvement of a genetic component in BPSD etiology seems beyond controversy, though the inconsistency of reported findings precludes a precise estimation of the significance of particular polymorphisms.

The *APOE* polymorphism is, to date, the only unanimously acclaimed genetic risk factor for the non-familial type of AD—harboring the *APOE* ε4 allele dose-dependently increases the risk of developing the disease, it is also associated with an earlier age

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Submitted: 08/12/11; Revised: 10/07/11; Accepted: 10/14/11
http://dx.doi.org/10.4161/pri.6.1.18428

at onset.[7] Apolipoprotein E plays a key role in the lipoprotein metabolism and cholesterol transport in plasma and the nervous system. ApoE seems to be implicated in various aspects of AD etiology: β-amyloid (Aβ) aggregation, deposition and clearance, neurofibrillary tangle formation, neurotoxicity, neuroinflammation, loss of synaptic plasticity and cholinergic dysfunction.[8]

The *CYP46* gene encodes cholesterol 24S-hydroxylase, an enzyme implicated in removing excessive brain cholesterol. Elevated concentration of cerebrospinal fluid 24S-hydroxycholesterol is one of the proposed biochemical markers of AD.[9] *CYP46* genotype can as well constitute a putative risk factor for AD. The studies so far have concentrated on the influence of an intronic C/T single nucleotide polymorphism (SNP) rs754203 on AD risk, however, with equivocal, inconclusive results.[10] In a study by our group, a new polymorphic site was discovered—a G to A change located in intron 2, 33 base pairs 5' of rs754203 (i2 SNP).[11]

The prion protein gene (*PRNP*) encodes for PrP^C, a glycoprotein causing Creutzfeldt-Jakob disease (CJD) and other prion diseases. *PRNP* codon 129 methionine (Met) or valine (Val) homozygosity is a known susceptibility factor for CJD.[12] *PRNP* genotype has also been implicated in the functioning of human long-term memory[13] and evaluated as a potential etiological factor in psychotic disorders.[14] The results of numerous studies on the influence of the *PRNP* genotype on the risk of AD were largely discordant. Nonetheless, in metaanalytic approach *PRNP* codon 129 homozygosity proved to be modestly, but significantly associated with AD risk (with an odds ratio of 1:3).[15]

The *PRND* gene, located close to the *PRNP* locus, encodes the protein called Doppel—the term is to emphasize its partial homology in amino acid sequence and a significant structural similarity to PrP^C. The open reading frame of *PRND* contains three polymorphic codons: 26, 56 and 174. Genetic polymorphisms in these three codons seem to be of little relevance for CJD risk.[16] The fourth polymorphic site is positioned in the 3' untranslated region (3'UTR) of the gene, 38 bases from codon 174.[17] The studies on the association between *PRND* codon 174 and AD risk produced divergent results.[18]

The aim of our study was to evaluate a possible association between the *APOE*, *CYP46*, *PRNP* and *PRND* genotypes and the profile of neuropsychiatric symptoms in the Polish AD and MCI subjects. To the best of our knowledge, the significance of *CYP46*, *PRNP* and *PRND* polymorphisms has never been studied in this context.

Results

The total sample consisted of 99 subjects with AD and 48 subjects with MCI. The median follow-up period was 32.5 ± 27.17 mo and 26.58 ± 20.63 mo, respectively. The demented participants were significantly older (76.63 ± 6.17 vs. 71.02 ± 6.61 years; $p < 0.001$) and less educated (9.68 ± 3.68 vs. 11.83 ± 4.13; $p < 0.001$) at baseline than their non-demented counterparts. Gender distribution was comparable in both groups (67.7% and 79.2% of females, respectively). The AD patients, by definition, performed significantly worse on cognitive tests, scoring less points on the MMSE (19.65 ± 4.63 vs. 27.6 ± 1.71; $p < 0.001$) and more on the CDR scale (1.34 ± 0.48 vs. 0.5; $p < 0.001$) compared with MCI subjects. The mean cognitive scores proved that most AD participants were in a mild-to-moderate stage of dementia at baseline. The majority of patients in both groups suffered from comorbid behavioral disturbances, however, the cumulative prevalence of behavioral symptoms was significantly higher in demented individuals (89.9% vs. 70.8% in the MCI group; $p = 0.007$). Not only the frequency, but also the level of behavioral burden inferred from the mean number of NPI symptoms occurring during the study period was more prominent in AD (4.19 ± 2.76) than in the MCI group (1.44 ± 1.27; $p < 0.001$), with a much higher ratio of subjects with at least four different behavioral symptoms present (54.5 vs. 8.3%; $p < 0.001$). The most prevalent behavioral disturbances in AD patients included irritability (62.6%), apathy (60.6%) and depression (49.5%), compared with anxiety, irritability and sleep problems (29.2% for all three) in MCI subjects. Apart from anxiety, elation and sleep change, all other NPI symptoms were significantly more frequent in the AD group. Baseline demographic and behavioral characteristics are summarized in **Table 1**.

The comparison of genotypic distribution and allele frequencies proved that the AD and MCI groups differed only in the prevalence of *APOE* ε4-containing genotypes. The proportion of *APOE* ε4-carriers was significantly higher in the demented individuals compared with MCI subjects (56.6 vs. 29.2%; $p = 0.003$). The distribution of polymorphisms in the *CYP46* (rs754203 and i2 new), *PRNP* (codon 129) and *PRND* genes (codons 26, 56, 174 and 3'UTR) was comparable between the study groups. All the evaluated genotypes did not deviate from the expected Hardy-Weinberg equilibrium. The specific data on genotypic distribution and allele frequencies are presented in **Table 2**.

Regarding genotype—phenotype correlations, the results are summarized in **Tables 3 and 4** (for AD and MCI participants, respectively), with statistically significant results presented in details. Among AD patients, the *APOE*, *CYP46*, *PRNP* and *PRND* codon 26, 56 and 174 polymorphisms were not associated with a particular behavioral phenotype. There was a trend for *PRND* codon 56 C/T heterozygosity to increase the risk for depression ($p = 0.08$). In contrast, several significant correlations were observed between the *PRND* 3'UTR polymorphism and NPI symptoms. Carrying the T allele turned out to be particularly harmful in terms of behavioral comorbidity, even after correcting for age, gender and the presence of *APOE* ε4 allele: AD patients bearing the T allele-genotype suffered an increased risk for delusions (RR = 6.6; 95% CI: 1.3–43.6; $p = 0.02$), anxiety (RR = 2.6; 95% CI: 1.2–6.8; $p = 0.02$), agitation/aggression (RR = 3.3; 95% CI: 1.4–10.2; $p = 0.01$), apathy (RR = 1.8; 95% CI: 1.2–3.0; $p = 0.02$), irritability/emotional lability (RR = 1.4; 95% CI: 1.0–2.4; $p = 0.05$), and aberrant motor behavior (RR = 2.9; 95% CI 1.1–12.4; $p = 0.05$). Furthermore, the possession of a *PRND* 3'UTR T-allele was significantly correlated not only with single NPI items, but also with a cumulative behavioral load – on average the carriers exhibited 5.11 ± 2.61 NPI symptom compared with 2.75 ± 2.24 in non-carriers ($p < 0.001$).

Table 1. Baseline demographic and behavioral characteristics of the study population

	AD (n = 99)	MCI (n = 48)	p value
Age, years (±SD)	76.63 ± 6.17	71.02 ± 6.61	<0.001
Female gender, n (%)	67 (67.7)	38 (79.2)	0.176
Education, years (±SD)	9.68 ± 3.68	11.83 ± 4.13	0.001
MMSE, points (±SD)	19.65 ± 4.63	27.6 ± 1.71	<0.001
CDR, points (±SD)	1.34 ± 0.48	0.5 (0)	<0.001
Behavioral symptoms during the study, n (%)	89 (89.9)	34 (70.8)	0.007
Mean number of NPI symptoms, n (±SD)	4.19 ± 2.76	1.44 ± 1.27	<0.001
Significant behavioral burden (>3 symptoms on the NPI), n (%)	54 (54.5)	4 (8.3)	<0.001
Delusions, n (%)	29 (29.3)	2 (4.2)	<0.001
Hallucinations, n (%)	21 (21.2)	0	<0.001
Agitation/aggression, n (%)	40 (40.4)	2 (4.2)	<0.001
Depression/dysphoria, n (%)	49 (49.5)	8 (16.7)	<0.001
Anxiety, n (%)	38 (38.4)	14 (29.2)	0.36
Elation/euphoria, n (%)	4 (4)	0	0.30
Apathy/indifference, n (%)	60 (60.6)	10 (20.8)	<0.001
Disinhibition, n (%)	13 (13.1)	1 (2.1)	0.03
Irritability/emotional lability, n (%)	62 (62.6)	14 (29.2)	<0.001
Aberrant motor behavior, n (%)	30 (30.3)	0	<0.001
Sleep and night-time behavior change, n (%)	40 (40.4)	14 (29.2)	0.25
Appetite and eating change, n (%)	29 (29.3)	4 (8.3)	<0.001

AD, Alzheimer disease; CDR, Clinical Dementia Rating; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory; SD, standard deviation.

The only statistically significant genotype-phenotype association for the MCI subjects was the reduction in the risk for night-time behavior change in *APOE* ε4 carriers (p = 0.039). No associations were observed for *CYP* rs754203, *CYP* i2 new polymorphisms, *PRNP* codon 129 genotype or *PRND* polymorphisms and NPI items. Non-significant trends could be demonstrated for some of the *PRND* genotypes: codon 56 C/T genotype increased the risk of sleep change (p = 0.07), codon 174 C/T heterozygosity had a protective effect on irritability (p = 0.07), while the 3'UTR C allele elevated the risk of concomitant anxiety (p = 0.07).

Discussion

In a carefully selected cohort of 99 AD and 48 MCI subjects, we demonstrated that the majority of cognitively impaired individuals suffered from comorbid behavioral changes. The overall level of behavioral pathology was related to the degree of cognitive decline, therefore the cumulative prevalence of behavioral symptoms and the level of behavioral burden (inferred from a mean number of NPI symptoms present during the study) turned out to be significantly higher in the AD group. Moreover, 9 of 12 individual NPI symptoms were more prevalent in AD compared with MCI subjects. Considering the genotype distributions, only the *APOE* ε4 allele frequency significantly differentiated AD from MCI subjects, with a significantly higher ratio of ε4 carriers in demented participants. The genotype and allelic frequencies in the studied *CYP46, PRNP* and *PRND* polymorphisms were comparable between study groups. However, the primary goal of the study was to evaluate the possible genotype-phenotype correlations. In the AD group, the *APOE*, *CYP46*, *PRNP* and *PRND* codon 26, 56 and 174 polymorphisms were not associated with a particular behavioral phenotype, while carrying the T allele of the *PRND* 3'UTR polymorphism significantly elevated the risk for comorbid delusions, anxiety, agitation/aggression, apathy, irritability/emotional lability and aberrant motor behavior. The associations remained equally robust (or even became stronger, this was the case for apathy and aberrant motor behavior) after controlling for potential confounders: age, gender and the presence of *APOE* ε4 allele, proving that the effects of *PRND* 3'UTR were independent of the *APOE* genotype. Harboring the *PRND* 3'UTR T-allele was also correlated with an increased cumulative behavioral load. In the MCI group, a reduction in the risk for night-time behavior change in *APOE* ε4 carriers was the only statistically significant observation. No associations were observed for the *CYP46*, *PRNP* or *PRND* polymorphisms and NPI items in MCI subjects, however, non-significant trends were demonstrated for some of the *PRND* genotypes (codon 56 C/T genotype increased the risk of sleep change, codon 174 C/T heterozygosity had a protective effect on irritability, 3'UTR C allele elevated the risk of anxiety).

In recent years, the importance of a genetic component in the multifactorial etiology of neuropsychiatric disorders has been accepted with increasing awareness. Genetic variance might not

Table 2. Genotypic distribution and allele frequencies of the studied polymorphisms

Genotype/allele frequencies, n (%)	AD (n = 99)	MCI (n = 48)	p value
***APOE*-ε4 dose**			
ε4 (-)	43 (43.4)	34 (70.8)	0.004
1 x ε4	46 (46.5)	10 (20.8)	
2 x ε4	10 (10.1)	4 (8.3)	
ε4 (-)	43 (43.4)	34 (70.8)	0.003
ε4 (+)	56 (56.6)	14 (29.2)	
***CYP46*-rs754203**			
C/C	18 (18.2)	7 (14.6)	0.55
C/T	41 (41.4)	17 (35.4)	
T/T	40 (40.4)	24 (50)	
C allele frequency	0.39	0.32	0.4
T allele frequency	0.61	0.68	
***CYP46*-i2 new**			
C/C	83 (83.8)	37 (77.1)	0.4
C/T	12 (12.1)	10 (20.8)	
T/T	4 (4.1)	1 (2.1)	
C allele frequency	0.9	0.875	0.8
T allele frequency	0.1	0.125	
***PRNP*-codon 129**			
M/M	37 (37.4)	19 (39.6)	0.96
M/V	51 (51.5)	24 (50)	
V/V	11 (11.1)	5 (10.4)	
M allele frequency	0.63	0.65	1.0
V allele frequency	0.37	0.35	
***PRND* AD (n = 64) MCI (n = 28)**			
Codon 26			
C/C	58 (90.6)	25 (89.3)	1.0
C/T	6 (9.4)	3 (10.7)	
Codon 56			
C/C	63 (98.4)	26 (92.9)	0.2
C/T	1 (1.6)	2 (7.1)	
Codon 174			
C/C	15 (23.4)	6 (21.4)	0.9
C/T	31 (48.5)	13 (46.4)	
T/T	18 (28.1)	9 (32.2)	
C allele frequency	0.48	0.45	1.0
T allele frequency	0.52	0.55	
3'UTR			
C/C	20 (31.2)	7 (25)	0.8
C/T	30 (46.9)	15 (53.6)	
T/T	14 (21.9)	6 (21.4)	
C allele frequency	0.55	0.52	1.0
T allele frequency	0.45	0.48	

AD, Alzheimer disease; MCI, mild cognitive impairment

only affect the risk of developing the disease, it may also have an impact on particular disease phenotypes or treatment results. Several genes have been evaluated for their hypothetical importance in BPSD pathogenesis, with an emphasis on *APOE* and genes coding for proteins involved in the process of neurotransmission. We have identified nearly 40 papers dealing with the influence of *APOE* genotype on BPSD risk in demented individuals (summarized in ref. 6). The results were unequivocal, a typical phenomenon in the field of psychiatric genetics: in the majority of studies the *APOE* genotype had no effect on behavioral disturbances,[19,20] in ~1/3 of published papers the *APOE* ε4 carriers suffered from an increased risk of non-cognitive dementia symptoms.[21] Only in 3 older papers by Holmes and colleagues, the *APOE* ε2 genotype, usually considered protective in terms of an overall AD risk, turned out to elevate the risk for comorbid depression and delusions.[22] In our cohort, carrying the *APOE* ε4 allele did not influence the presence of behavioral symptoms in AD patients, in line with the substantial proportion of available data. However, in the MCI group it reduced the risk for sleep disturbances. To date, only two studies evaluated the relevance of the *APOE* genotype to sleep quality in AD subjects. In the large, case-control association study no relationship was noticed between the *APOE* genotype and sleep change—neither the presence nor absence of the *APOE* ε4-containing alleles had any significant independent relationship with sleep when analyzed separately.[23] However, in a longitudinal study conducted on a much smaller population of 44 AD patients, the *APOE* status was associated with the progression of sleep/wake disturbances, with an overall greater deterioration on sleep parameters in patients negative for the ε4 allele.[24] The rationale for this phenomenon is probably unrelated to the deposition of neuropathologic changes distinctive of AD, as neurofibrillary tangles and amyloid plaques are not typically seen in the suprachiasmatic nucleus-pineal axis.[25]

The most consistent observation in our cohort was the behaviorally detrimental effect of the T-allele in 3'UTR *PRND* polymorphism. Given our insufficient knowledge about *PRND* and the Doppel protein, it is hardly possible to provide a plausible biological rationale for this finding. The 3'UTR polymorphism is a non-coding C/T change, therefore unlikely to affect Doppel protein structure.[17] Considering its proximity to codon 174 polymorphism, it is unsurprising these two are in linkage disequilibrium (LD). However, the significance of codon 174 methionine-to-threonine substitution in modifying the predisposition for neurodegenerative disorders is dubious at best,[18,26,27] the polymorphism does not seem to influence the Doppel structure as well.[17] Therefore, if the LD phenomenon was to account for the clinical significance of the 3'UTR T-allele in our AD patients, other polymorphisms close to 3'UTR would have to be involved. Moreover, in the adult human brain Doppel is expressed in minute concentrations, primarily in the cerebellum.[28] Although its immunoreactivity in dystrophic neurites of senile plaques in AD has also been observed, the clinical significance of this finding is unknown.[28] The overexpression of Doppel in the brain has only been observed in *PRNP*-knockout mice, leading to the degeneration of cerebellar Purkinje cells and clinical ataxia.[29] No other phenotypic, behavioral changes were observed. Considering the

Table 3. Associations between genetic polymorphisms and neuropsychiatric symptoms in Alzheimer disease

Polymorphism	BPSD symptom in AD, n (%)		Statistics
No significant associations between *APOE* ε4 presence or dose and NPI items			
No significant associations between *CYP* rs754203 or *CYP* i2 new polymorphisms and NPI items			
No significant associations between *PRNP* codon 129 polymorphism and NPI items			
No significant associations between *PRND* codons 26, 56, 174 polymorphisms and NPI items • a trend for *PRND* codon 56 C/T genotype to increase depression risk; p = 0.08			
PRND* 3'UTR and delusions			
***PRND* 3'UTR**	**Delusions present (n = 17)**	**Delusions absent (n = 47)**	
C/C	1 (5.9)	19 (40.4)	
C/T	12 (70.6)	18 (38.3)	p = 0.015
T/T	4 (23.5)	10 (21.3)	
T allele present	16 (94.1)	28 (59.6)	RR = 6.6 (95% CI 1.3–43.6)
T allele absent	1 (5.9)	19 (40.4)	p = 0.02
PRND* 3'UTR and anxiety			
***PRND* 3'UTR**	**Anxiety present (n = 28)**	**Anxiety absent (n = 36)**	
C/C	4 (14.3)	16 (44.4)	
C/T	17 (60.7)	13 (36.1)	p = 0.03
T/T	7 (25)	7 (19.5)	
T allele present	24 (85.7)	20 (55.6)	RR = 2.6 (95% CI 1.2–6.8)
T allele absent	4 (14.3)	16 (44.4)	p = 0.02
PRND* 3'UTR and agitation/aggression			
***PRND* 3'UTR**	**Agitation present (n = 26)**	**Agitation absent (n = 38)**	
C/C	3 (11.55)	17 (44.7)	
C/T	16 (61.55)	14 (36.8)	p = 0.02
T/T	7 (26.9)	7 (19.5)	
T allele present	23 (88.45)	21 (55.3)	RR = 3.3 (95% CI 1.3–10.2)
T allele absent	3 (11.55)	17 (44.7)	p = 0.01
PRND* 3'UTR and apathy			
***PRND* 3'UTR**	**Apathy present (n = 40)**	**Apathy absent (n = 24)**	
C/C	8 (20)	12 (50)	
C/T	23 (57.5)	7 (29.2)	p = 0.03
T/T	9 (22.5)	5 (20.8)	
T allele present	32 (80)	12 (50)	RR = 1.8 (95% CI 1.2–3.0)
T allele absent	8 (20)	12 (50)	p = 0.02
PRND* 3'UTR and irritability/emotional lability			
***PRND* 3'UTR**	**Irritability present (n = 47)**	**Irritability absent (n = 17)**	
C/C	11 (23.4)	9 (52.9)	
C/T	25 (53.2)	5 (29.4)	p = 0.08
T/T	11 (23.4)	3 (17.7)	
T allele present	36 (76.6)	8 (47.1)	RR = 1.4 (95% CI 1.0–2.4)
T allele absent	11 (23.4)	9 (52.9)	p = 0.05

*controlled for age, gender and the presence of *APOE* ε4 allele. AD, Alzheimer disease; BPSD, behavioral and psychological symptoms of dementia; NPI, Neuropsychiatric Inventory.

Table 3. Associations between genetic polymorphisms and neuropsychiatric symptoms in Alzheimer disease

Polymorphism	BPSD symptom in AD, n (%)		Statistics
PRND* 3'UTR and aberrant motor activity (AMB)			
***PRND* 3'UTR**	**AMB present (n = 17)**	**AMB absent (n = 47)**	
C/C	2 (11.8)	18 (38.3)	
C/T	8 (47)	22 (46.8)	p = 0.04
T/T	7 (41.2)	7 (14.9)	
T allele present	15 (88.2)	29 (61.7)	RR = 2.9 (95% CI 1.1–12.4)
T allele absent	2 (11.8)	18 (38.3)	p = 0.05

*controlled for age, gender and the presence of *APOE* ε4 allele. AD, Alzheimer disease; BPSD, behavioral and psychological symptoms of dementia; NPI, Neuropsychiatric Inventory.

Table 4. Associations between genetic polymorphisms and neuropsychiatric symptoms in mild cognitive impairment

Polymorphism	BPSD symptom in MCI, n (%)		Statistics
***APOE* ε4 and sleep/night-time behavior change**			
***APOE* ε4**	**Sleep problems present (n = 14)**	**Sleep problems absent (n = 34)**	
ε4 present	1 (7.1)	13 (38.2)	p = 0.04
ε4 absent	13 (92.9)	21 (61.8)	
No significant associations between *CYP* rs754203 or *CYP* i2 new polymorphisms and NPI items			
No significant associations between *PRNP* codon 129 polymorphism and NPI items			
No significant associations between *PRND* polymorphisms and NPI items			
• a trend for *PRND* codon 56 C/T genotype to increase the risk of sleep change; p = 0.07			
• a trend for *PRND* codon 174 C/T genotype to protect from irritability; p = 0.07			
• a trend for *PRND* 3'UTR C allele to increase the risk of anxiety; p = 0.07			

BPSD, behavioral and psychological symptoms of dementia; MCI, mild cognitive impairment; NPI, Neuropsychiatric Inventory.

cerebellar selectivity of Doppel in the CNS and the obscurity of the mechanisms involved, its association with AD in general or with specific AD symptomatology remains vague.

No other significant genotype-phenotype correlations were observed with the two *CYP46* polymorphisms, *PRNP* codon 129 and other *PRND* polymorphisms. Discussing their putative relevance for cognitive decline or BPSD is beyond the scope of this report. The strengths of the study include a long follow-up period as well as prospective behavioral evaluation. Cross-sectional studies can omit episodes that occur outside the assessment period. With a longitudinal design, a higher frequency of symptoms can be detected, significantly influencing the attribution of patients to predefined study groups. The small size of the study population constitutes a major limitation of the study.

Methods

Subjects. We studied 99 patients with AD diagnosed according to the NINCDS-ADRDA criteria[30] and 48 subjects with MCI diagnosed according to the criteria by Petersen[31] recruited at the Department of Old Age Psychiatry and Psychotic Disorders, Medical University of Lodz, Poland. Since the goal of this analysis was to test the association of abnormal behaviors with candidate genes in patients with cognitive disturbance, genotype comparisons with healthy controls are not presented. All patients participating in the study underwent a comprehensive evaluation, including cognitive assessment, medical history, physical and neurological examinations, complete blood count, serum chemistries and brain CT or MRI. The clinical diagnosis was corroborated by an experienced neuropsychologist. Subjects were excluded for any neurological or medical disorder other than AD potentially accounting for the cognitive decline, or for significant psychiatric illness prior to the onset of cognitive deterioration, alcohol or substance abuse. The Clinical Dementia Rating (CDR) scale[32] and the Mini Mental State Examination (MMSE),[33] were applied to assess the severity of cognitive impairment. All patients had nonprofessional regular caregivers and were living in the community.

The patient and the caregiver were thoroughly interviewed about behavioral disturbances occurring after the onset of cognitive decline and before study entry. The presence and profile of BPSD were evaluated at baseline and prospectively during follow-up with the caregiver-rated Neuropsychiatric Inventory (NPI),[34] the assessment was repeated at least every 6 months. In the available literature the NPI was the most widely employed scale evaluating behavioral symptoms in dementia patients, including research on behavioral genetics in AD subjects. Thus, the choice of this instrument for BPSD profile assessment seems reasonable and well justified as well as allowing for between-study comparisons. The NPI comprises the following 12 behavioral domains:

delusions, hallucinations, agitation/aggression, depression, anxiety, elation/euphoria, apathy/indifference, disinhibition, irritability/lability, aberrant motor behavior, sleep disturbances and eating disturbances. For each of the symptoms the caregiver is confronted with a set of screening questions to establish if the symptom of interest has ever been present. If the answer to any of them is positive, the frequency (1–4 points) and severity (1–3 points) of each of the individual BPSD items is rated afterwards and the frequency x severity final score is calculated (0–12 points for every item; 0–144 points for the whole scale). Furthermore, the caregiver evaluates the level of distress associated with each of the symptoms, from 0 (no stress) to 5 (extreme stress) points. In the field of dementia behavioral genetics three major NPI-related methodological strategies are commonly used. If the study is planned to focus on particular symptoms, e.g., psychosis or depression, different predefined cut-off scores (frequency x severity) are sometimes employed to evaluate whether the putative genotype-phenotype correlations are influenced by the symptom's level of clinical significance.[21] In the second variant a given cut-off score (or different scores for different symptoms) can be considered one of the inclusion criteria to only recruit patients with clinically meaningful symptoms.[19] However, in the majority of studies, the participants are simply dichotomized into those having ever experienced a particular symptom at any time and those who did not over the whole follow-up period.[20] In our work, we followed the third scenario—only the presence/absence of the 12 BPSD items were rated (frequency x severity ≥ 1), the individual and overall final scores were not recorded. The behavioral symptoms considered irrelevant by the caregiver (zero points on the distress subscale) were not marked as present to minimize the possibility of spurious statistical findings.

Some medications received by subjects at the time of evaluation and during the study, particularly cholinesterase inhibitors, memantine and psychotropic drugs may have potentially impacted behavioral variables analyzed, primarily via preventing their emergence. The entire study population was of eastern European (Caucasian) descent. All patients (or patients' relatives) provided an informed consent. The study was performed according to the Declaration of Helsinki with the approval of the Ethics Committee of the Medical University of Lodz.

Genotyping. Genotyping was performed at the Department of Molecular Pathology and Neuropathology, Medical University of Lodz, Poland. Laboratory personnel performing the genetic analyses were blinded for sample identity. Genomic DNA was isolated from leukocyte-rich interphase layer of EDTA-anticoagulated blood by the phenol-chloroform method, dissolved in nuclease-free water and stored at 4°C pending assay. The *APOE* genotype was established by restriction fragment length polymorphism analysis according to Chapman et al. A 165 bp fragment of *CYP46* intron 2 was amplified with a polymerase chain reaction (PCR) using the primers specified by Papassotiropoulos et al.[36] and PCR protocol described by Golanska et al.[11] A 925-bp fragment of *PRND* gene, including the coding sequence and adjacent DNA regions, was isolated according to the protocol described by Golanska et al.[18] Sequencing of *CYP46* and *PRND* PCR products was performed using a Li-Cor automated laser fluorescence sequencer. The codon 129 polymorphism of the *PRNP* gene was evaluated by isolating the 755-bp fragment containing the coding sequence amplified by the PCR reaction with primers specified by Golanska et al.[18] The PCR product was digested with *Mae*II or *Nsp*I restriction endonucleases, the resulting DNA fragments were separated on a 2% agarose gel.

Statistical methods. Genotype-phenotype correlations were examined using Student's t-test for continuous variables or Pearson's χ^2 test for dichotomous variables. χ^2 analysis was also employed to test whether genotype frequencies deviated from the expected Hardy-Weinberg equilibrium. For 2 x 3 contingency tables, due to small frequencies in some cells, the Freeman-Halton extension of the Fisher exact test was used instead of the χ^2 test. Logistic regression analysis was performed to assess Odds Ratio (OR), adjusting for possible confounding variables (age, gender, *APOE* genotype). For all comparisons, values of $p < 0.05$ were considered statistically significant. The statistical analyses were performed with the SPSS software (SPSS Inc.).

Conclusions

The inconsistency of the results is one of the major obstacles in the field of psychiatric genetics. One has to bear in mind the potential sources of bias leading to non-replication. These include: recruitment process based solely on symptomatic, biologically undetermined criteria; different diagnostic criteria employed; variability in the choice and definition of symptoms; evaluating carrier status vs. allele dose; selection bias; inadequate statistical power; finally, inherent limitations of complex traits' genetics—multifactorial etiology, weak effects of individual polymorphisms, gene-gene and gene-environment interactions. Acknowledging the fact that the significance of *CYP46*, *PRNP* or *PRND* polymorphisms in BPSD etiology has not been investigated before, and considering abundant but conflicting data on *APOE* and AD endophenotypes, future studies on much larger populations are necessary for a precise estimation of their true relevance for AD, MCI and BPSD.

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