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Polymorphisms in the apolipoprotein E (APOE) gene in gerontopsychiatric patients

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Abstract Two recently described polymorphisms in the promoter region of the apolipoprotein E (APOE), the –491A/T and Th1/E47csT/G polymorphism, have been suggested to be associated with an increased risk for Alzheimer's disease (AD) independent from the APOE ε4 carrier status. We studied the association between the APOE ε4 polymorphism and the –491A/T and Th1E47csT/G polymorphisms in a sample of 118 healthy, non-demented controls and 239 consecutively recruited gerontopsychiatric patients diagnosed as: Alzheimer's disease (N = 89), age mild cognitive impairment (N = 32), memory complainers without any cognitive deficit (N = 54) and depression/other psychiatric disorders (N = 64), to test whether the investigated polymorphisms have a high enough selectivity and specificity to distinguish between the different gerontopsychiatric disorders or to differentiate AD genetically from other forms of dementia, respectively. Also a possible association with the APOE ε4 polymorphism was examined. We found a statistically significant association between the APOE ε4 allele and Alzheimer's disease (p = 0.0001) and age associated memory impairment (p = 0.006). Our study failed to show an association between the promoter polymorphisms –491A/T and Th1E47csT/G in the APOE gene and gerontopsychiatric disorders either alone or in relationship to the APOE ε4 polymorphism. However, if we combine our results with three previous published positive reports there seems to be an association between the –491A/T polymorphism and AD, though its size is less than found in the original publication.

Key words Alzheimer's disease · Apolipoprotein E · Promoter polymorphisms · Association study

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Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly and exists in familial and sporadic forms. Mutations in the genes for the β-amyloid precursor protein (Goate et al. 1991), presenilin 1 (Sherrington et al. 1995) and presenilin 2 (Levy-Lahad et al. 1995) lead to the relatively rare autosomal dominant forms of early onset familial AD. However, the majority of patients with AD show a late age of onset and a complex pattern of inheritance in which genetic and non-genetic factors are likely to be interacting. The ε4 allele of the apolipoprotein E (APOE) gene on chromosome 19 is associated with an increased risk of developing AD in all ethnic groups, across all ages between 40 and 90 years (Corder et al, 1993). In contrast, the ε2 allele seems to have a protective effect against AD (Corder et al. 1994). However, many AD cases have no APOE ε4 alleles and likewise some carriers of the APOE ε4 allele do not develop AD. The biological mechanism by which the ε4 allele might promote increased risk for AD is still unclear. It might be possible that the ε4 allele is a genetic marker in linkage disequilibrium with a second nearby sequence variation either in the APOE gene or in another neighboring gene. Because of the fact that the levels of APOE mRNA in AD brains and APOE protein in the plasma of AD patients is increased (Yamada et al. 1995; Taddei et al. 1997) compared to controls several studies have performed mutation analysis within the regulatory promoter region of the APOE gene. Bullido et al. (1998) demonstrated that a common polymorphism (–491A/T) was associated with an increased risk of developing AD independent from the APOE ε4 status. This result was confirmed by Lambert et al. (1998), who found an additional association with a second polymorphism in the promoter region of the APOE gene (Th1E47csT/G), but these results are still inconclusive (Song et al. 1998; Toji et al. 1999; Roks et al. 1998). These two polymorphisms seem to have functional effects on the transcriptional activity of the APOE gene in this form that the A-allele

of the –491A/T polymorphism and the T-allele of the Th1E47csT/G polymorphism leads to an increased production of APOE protein and therefore to an increased risk of developing AD (Artiga et al. 1998).

In the current study we investigated the APOE ε4 polymorphism and the described two polymorphisms in the regulatory region of the APOE gene (–491A/T, Th1E47csT/G) alone and also in combination with the APOE ε4 allele distribution in a gerontopsychiatric patient sample consisting of the following four diagnostic subgroups: Alzheimer's disease (AD; N = 89), mild cognitive impairment (MCI; N = 32), memory complainers without any cognitive deficit (MC; N = 54) and depression/other psychiatric disorders (DOP; N = 64). The aim of this study was to test, in the case of an association, whether the analyzed polymorphisms have a high enough specificity and selectivity to distinguish between the different gerontopsychiatric subgroups, or to differentiate AD genetically from other forms of dementia, respectively.

Subjects and methods

Sample

239 consecutively recruited gerontopsychiatric patients were included (110 males, 129 females, mean age 67 ± 12 years). Diagnosis of dementia was performed according to DSM-IV criteria. Cognitive testing by neuropsychological evaluation was performed in all patients according to MMSE (Folstein et al. 1975), CERAD battery (Welsh et al. 1994), multiple choice vocabulary test (Schmidt and Metzler 1989) and a variant of the trail making test (Oswald and Roth 1989). The gerontopsychiatric patients were diagnosed as follows:

Alzheimer's disease (AD), N = 89, 39 males, 50 females, mean age 73 ± 9 years, according to NINCDS-ADRDA criteria (McKhann et al. 1984). Mild cognitive impairment (MCI), N = 32, 17 males, 15 females, mean age 67 ± 10 years was defined by neuropsychological evaluations (Petersen et al. 1997), (MMSE-score ≥ 25, CERAD word-list memory-score ≥ 12, CERAD word-list recall (delay)-score ≥ 3). Memory complainers without any cognitive deficit (MC), N = 54, 22 males, 32 females, mean age 61 ± 10 years. MC was defined by neuropsychological evaluations (MMSE score ≥ 25, CERAD word-list memory-score ≥ 12, CERAD word-list recall (delay)-score ≥ 3). Depression and other psychiatric disorders (DOP), N = 64, 32 males, 32 females, mean age 63 ± 12 years. Diagnosis of DOP was defined according to ICD–10 criteria.

The control group consisted of 118 healthy, non-demented, unrelated Caucasians from the general population in southern Germany (no hospital staff) matched for age and gender (55 males, 63 females, mean age 47 ± 12 years). All probands of the control group were screened psychiatrically using personality questionnaires.

Genotyping

Genomic DNA was extracted from leukocytes or whole blood according to standard procedures. APOE genotype was determined according to Wenham et al. (1991). The genotyping of the –491A/T and Th1E47csT/G polymorphisms was performed by fluorescence detection on the Light Cycler System (Roche Diagnostics) with the following conditions:

–491A/T: forward primer: 5'ACG CCT GGC TAA CTT TTG T–3'; reverse primer: 5'-ATG AAT GTA ATC TGG AGA GGG G–3'; acceptor hybridization probe: 5'-LCRed640-TTC GCC CAC TGT GGC CTC CCA AAG TG-phosphate; donor hybridization probe: 5'-CTG GTC TCA AAC TCC TGA CCT TAA GT-fluorescein; Th1E47csT/G: forward primer: 5'-CAG GAA AGG ACA GGG TCA GG–3'; reverse primer: 5'-AGT AGG ACT CAA GGA TCC CAG–3'; acceptor hybridization probe: 5'-LCRed640-TGT CCT CCC TTC CTG GGG ACT GT-phosphate; donor hybridization probe: 5'-AGG GTG TCT GTA TTA CTG GGC GA-fluorescein.

PCR was performed with 50 ng DNA according to the manufacture's instructions for 40 cycles of denaturation (95 °C, 0 s, ramp rate 20 °C/s), annealing (55 °C, 10 s, ramp rate 20 °C/s) and extension (72 °C, 10 s, ramp rate 20 °C/s). After amplification a melting curve was generated by holding the reaction at 40 °C for 45 s and then heating slowly to 95 °C with a ramp rate of 0.1 °C/s. The fluorescence signal was plotted against temperature to give melting curves for each sample.

Laboratory procedures were carried out blind to case-control status.

Statistical analysis

Genotype and allele frequencies between patients and controls were compared by using exact tests or χ^2 tests, if appropriate. Odds ratios were calculated for risk evaluation. Power calculations were based on the results of the studies by Bullido et al. (1998) and Lambert et al. (1998). The interaction of APOE with the two promoter polymorphisms was assessed with a logistic regression analysis.

Results

We analyzed the genotypes of the APOE polymorphism and of the APOE promoter polymorphisms –491A/T and Th1E47csT/G in a gerontopsychiatric patient sample divided into the four different diagnostic subgroups: Alzheimer's disease (N = 89), mild cognitive impairment (N = 32), memory complainers without any cognitive deficit (N = 54) and depression/other psychiatric disorders (N = 64).

Table 1 shows genotype and allelic distribution of the APOE ε4 allele in patients and controls. As expected, we found a significant association between the APOE ε4 allele and the AD group (p = 0.0001, OR = 2.5, 95 % CI 1.6–4.1, two sided Fisher's exact test). Interestingly a significant association was also found with the MCI group (p = 0.006, OR 2.5, 95 % CI 1.3–4.8, two sided Fisher's exact test). The distribution of the APOE ε4 allele in the other two patient subgroups, MC and DOP, showed no significant differences compared to the control group.

The distribution of the APOE promoter polymorphisms –491A/T and Th1E47csT/G followed in all

Tab. 1 Genotype and allelic distribution of the APOE ε 4 allele

	Genotypes			Allele frequency
Sample	4/4	4/–[a]	–/–[b]	ε4
AD (N = 89)	5	46	38	**0.31***
MCI (N = 32)	2	16	14	**0.31***
MC (N = 54)	0	24	30	0.22
DOP (N = 64)	0	26	38	0.20
Controls (N = 118)	2	32	84	**0.15***

[a] one ε4 allele, [b] no ε4 allele

* AD/Controls: Alleles: p = 0.0001 (OR = 2.5, 95 % CI 1.6–4.1, Fisher's exact test, two sided); Genotypes: p = 0.0002, χ^2 = 17.42, df = 2.

* MCI /Controls: Alleles: p = 0.006 (OR = 2.5, 95 % CI 1.3–4.8, Fisher's exact test, two sided); Genotypes: p = 0.01, χ^2 = 8.98, df = 2

Tab. 2 Genotype and allelic distribution of the –491A/T polymorphism

Sample	Genotypes[a]			Allele frequency[b]	
	T/T	A/T	A/A	T	A
AD (N = 89)	1	20	68	0.12	0.88
MCI (N = 32)	2	6	24	0.16	0.84
MC (N = 54)	0	19	35	0.18	0.82
DOP (N = 64)	5	21	38	0.24	0.76
Controls (N = 118)	5	33	80	0.18	0.82

[a, b] Not significant

Tab. 3 Genotype and allelic distribution of the Th1E47csT/G polymorphism

Sample	Genotypes[a]			Allele frequency[b]	
	G/G	G/T	T/T	G	T
AD (N = 89)	16	51	22	0.47	0.53
MCI (N = 32)	7	14	11	0.44	0.56
MC (N = 54)	19	24	11	0.57	0.43
DOP (N = 64)	14	35	15	0.49	0.51
Controls (N = 118)	31	64	23	0.54	0.47

[a, b] Not significant

groups the Hardy-Weinberg equilibrium. Tables 2 and 3 shows genotype and allelic distributions of the –491A/T and Th1E47csT/G polymorphisms in patients and controls. For both polymorphisms no significant difference in the allele or genotype frequencies was found between any of the clinical groups and the control group. Regarding the Th1E47csT/G polymorphism the difference in the frequency of the T allele between AD and controls was 6 % (53 % vs. 47 %, OR = 1.31), with the T allele being more frequent in the AD group. For the –491A/T polymorphism the difference in the frequency of the A allele between AD and controls was 6 % (88 % vs. 82 %, OR = 1.58), the A allele being more frequent in the AD group. The size of the allele difference is much less than in the Bullido et al. (1998) Spanish sample (13 %, OR = 2.38), and even less than in their US sample (8 %, OR = 1.87). Our sample size was large enough to replicate the effect, if it would be in this size: assuming an allele frequency of 0.80 in the control sample, a difference of 0.10 to the Alzheimer sample and $\alpha = 0.05$, our sample size of 89 (AD) and 118 (control) had a power of 0.82 to detect this difference.

To test whether an association exists with the APOE ε4 polymorphism in one of the investigated patient subgroups, we compared the allele frequencies of the two promoter polymorphisms –491A/T and Th1E47csT/G with the APOE ε4 carrier- and APOE ε4 non-carrier status. The allelic distributions did not significantly differ between the patient subgroups and the controls for all of the analyzed polymorphisms studied, so that the combined analysis of the two promoter polymorphisms with the APOE ε4 allele carrier and non-carrier status also led to negative results. Tables 4 and 5 show the genotype and

Tab. 4 Genotype and allelic distribution of the combination –491A/T – APOE ε4 polymorphism

APOE	Sample	Genotypes[c]			Allele frequency[d]	
		T/T	A/T	AA	T	A
4/4 and 4/–[a]	AD (N = 51)	0	8	43	0.08	0.92
	MCI (N = 18)	1	1	16	0.08	0.92
	MC (N = 24)	0	12	12	0.25	0.75
	DOP (N = 26)	4	8	14	0.31	0.69
	Controls (N = 34)	0	8	26	0.12	0.88
–/–[b]	AD (N = 38)	1	12	25	0.18	0.82
	MCI (N = 14)	1	5	8	0.25	0.75
	MC (N = 30)	0	7	23	0.12	0.88
	DOP (N = 38)	1	13	24	0.20	0.80
	Controls (N = 84)	5	25	54	0.21	0.79

[a] 4/–: one ε 4 allele, [b] –/–: no ε 4, [c, d] Not significant

Tab. 5 Genotype and allelic distribution of the combination Th1E47csT/G – APOE ε4 polymorphism

APOE	Sample	Genotypes[c]			Allele frequency[d]	
		G/G	G/T	T/T	G	T
4/4 and 4/–[a]	AD (N = 51)	6	30	15	0.41	0.59
	MCI (N = 18)	2	11	5	0.42	0.58
	MC (N = 24)	8	10	6	0.54	0.46
	DOP (N = 26)	6	13	7	0.48	0.52
	Controls (N = 34)	2	23	9	0.40	0.60
–/–[b]	AD (N = 38)	10	21	7	0.54	0.46
	MCI (N = 14)	5	3	6	0.46	0.54
	MC (N = 30)	11	14	5	0.60	0.40
	DOP (N = 38)	8	22	8	0.50	0.50
	Controls (N = 84)	29	41	14	0.59	0.41

[a] 4/–: one ε 4 allele, [b] –/–: no ε 4 allele, [c, d] Not significant

the allelic distribution for the combination of the APOE ε4 allele carriers and non-carriers and the –491A/T and Th1E47csT/G polymorphisms. We tested the possible combined influence of the two promoter polymorphisms (–491AA vs. –491AT/TT and Th1E47csTT vs. Th1E47csTG/GG) on the APOE polymorphism (ε4 present vs. not present) with a multiple logistic regression analysis. The odds ratio for APOE (unadjusted OR = 3.32, $p < 0.0001$) did not change significantly after adjusting for the effects of the two promoter polymorphisms (adjusted OR = 3.15, $p < 0.0001$). Adjusting the ORs of the promoter polymorphisms for the presence of APOE ε4 also had no effect.

To test whether our data are false negative results due to the relatively young control group compared to the patient groups we investigated the allele frequencies in two subgroups (controls > 60 years and controls < 60 years, data not shown). There were no significant differences in the allelic distribution.

Discussion

Recently, several findings about an association between polymorphisms in the promoter region of the APOE gene and AD have been reported (Bullido et al. 1998; Lambert et al. 1998). These results seemed to be confirmed by physiological data that differences in the allelic expression of the APOE gene were observed (Laws et al. 1999). We analyzed the genotypes of the APOE ε4 polymorphism and the APOE promoter polymorphisms –491A/T and Th1E47csT/G in a gerontopsychiatric patient sample diagnosed as AD, MCI, MC and DOP. It is certainly disputable that depression/other psychiatric disorders represents very heterogeneous group, but under the assumption that DOP have a different etiology than AD we also included this group in the study.

Our study confirms the association between Alzheimer's disease and the ε4 allele of the APOE gene. Furthermore we observed a significant increase of the APOE ε4 allele in the mild cognitive impairment group, a finding which could support the hypothesis that MCI is considered as a preclinical phase of AD (Parnetti et al. 1996).

According to our preliminary results the APOE ε4 allele is apparently not a risk factor in the gerontopsychiatric patient subgroups MC and DOP, so that these symptoms, which are frequently observed in gerontopsychiatric patients, might be related to other still unknown biological, genetic or environmental factors.

The association of the two promoter polymorphisms of the APOE gene –491A/T and Th1E47csT/G with AD is less clear. We did not find a significant association between –491A/T and AD. In our data the preponderance of the A allele frequency in AD compared to controls (6 %) was much less than in the Bullido et al. (1998) Spanish sample (13 %, OR = 2.38), and even less than in their US sample (8 %, OR = 1.87). Our sample size was large enough to replicate the effect, if it would be in this size: assuming an allele frequency of 0.80 in the control sample, a difference of 0.10 to the Alzheimer sample and $\alpha = 0.05$, our sample size of 89 (AD) and 118 (control) had a power of 0.82 to detect a difference in this size. The same polymorphism was also studied by Ahmed et al. (1999) with 192 AD patients and 106 controls, where the reported difference was 11 % (OR = 2.54, $p = 0.01$) in the same direction. If we combine these four samples (including ours) there seems to be an association (the mean preponderance of the A allele frequency in the AD groups compared to controls being 9 %), though its size is less than originally found in the Spanish sample of Bullido et al. (1998).

Also, we did not find a significant association between Th1E47csT/G and AD. Nevertheless, the direction and amount of the difference in the T allele frequency is exactly the same as in the paper of Lambert et al. (1998), though not significant due to the smaller sample size in our study. Taken together the T allele seems to be more frequent in AD (53 %) than in controls (47 %), though the effect size is relatively small. With such small effect sizes, the possibility of rival hypotheses (linkage disequilibrium, diagnostic subgroup effects, ethnic group effects) is relatively high.

The two promoter polymorphisms are independent from the APOE ε4 carrier status. This could be demonstrated by subgroup analysis as well as by multiple logistic regression and replicates results of Bullido et al. (1998) and Lambert et al. (1998).

We did not find an association of the two promoter polymorphisms with the other neuropsychiatric disorders studied, i. e., MCI, MC and DOP.

In summary, our data about APOE ε4 allele distribution in patients with Alzheimer's dementia are consistent with results described in the literature and add information concerning an increased APOE ε4 allele frequency in patients with MCI. The APOE gene promoter polymorphisms –491A/T and Th1E47csT/G seem to be independent risk factors for AD, though both of considerably less impact than APOE.

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