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Neuroscience Letters 400 (2006) 187–190
Neuroscience Letters
www.elsevier.com/locate/neulet

α2-Macroglobulin, lipoprotein receptor-related protein and lipoprotein receptor-associated protein and the genetic risk for developing Alzheimer's disease

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Received 16 October 2005; received in revised form 30 December 2005; accepted 16 January 2006

Abstract

α2-Macroglobulin (α2M) as well as its receptor, the low-density lipoprotein receptor-related (LRP) and the receptor-associated protein (RAP) are involved in the clearance of cerebral Aβ. Current evidence suggests that polymorphisms in the genes of α2M, LRP and RAP may have functional effects on the proteins. Two independent association samples of 271 AD patients and 280 representative controls were investigated whether the risk for developing AD is altered in carriers of polymorphisms in the α2M-gene (Va1000Ile), in the LRP-gene (Ala216Val) and in the RAP-gene (Val311Met). Genotypes were determined by standard PCR and restriction fragment length polymorphism. The results were adjusted for age, gender and apolipoprotein E-ε4 (*APOE*) polymorphism. Inheritance of α2M conferred a small increased risk for sporadic AD with an estimated Mantel–Haenszel odds ratio of 1.47. There was no age- or gender-dependent increase in α2M Val1000Ile allele frequencies in AD patients compared to controls. There was no significant difference in the allele frequencies among control and AD subjects for the LRP and RAP polymorphisms. We found no evidence of an interaction between the α2M and RAP or LRP with regard to conferred risk. Our data suggest that the α2M Val1000Ile polymorphism is weakly associated with AD. Although LRP as well as RAP seem to play an essential role in the metabolism of α2M and *APOE*, there is no increase in the genetic risk for AD in patients carrying the investigated polymorphisms.

Keywords: Dementia; Amyloid; Inflammation; Lipoprotein; Polymorphism

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder leading to loss of reasoning, orientation and memory. Linkage analyses and association studies have identified several genetic factors that cause or predispose carriers to AD. One established risk factor is *APOE*. Thus, variation of the *APOE* locus accounts for at most 50% of the genetic variation to develop the disorder, however, there must be other genetic risk variants that account for the remaining risk [19].

α2M is a plasma protease inhibitor (720 kDa) with a large variety of functions [3]. In the brain, α2M was associated with immune-mediated CNS diseases [1,18] and has been co-localized immunohistochemically to β-amlyoid (Aβ) plaques [20], a requisite neuropathological features of AD. In vitro, fibril formation and neurotoxicity of Aβ are attenuated through forming stable complexes with α2M [6,16]. Two polymorphisms in the α2M gene have been associated with AD [2,5,12], however, others have failed to repeat the initial reports [4,21,22].

The multifunctional endocytic receptor LRP mediates the catabolism of a number of molecules known to be important for the pathogenesis of AD, including proteases, protease inhibitor complexes, lipoproteins (predominantly *APOE* containing lipoproteins) and α2M [18]. In AD, LRP plays an impor-

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doi:10.1016/j.neulet.2006.01.040

tant role in the clearance pathway of Aβ [8,11,16]. Furthermore, the plaque density was significantly altered as a consequence of different LRP genotypes in postmortem AD patients [10]. It also mediates the *APOE*-effects on neurite outgrowth and development [17]. The binding of all currently known ligands to LRP is inhibited by RAP [11,23]. RAP acts as a physiological chaperone for LRP and is required for normal functional expression of LRP within the secretory pathway [23]. Several polymorphisms have been found in the RAP gene [13].

In this report we examined a large cohort of German AD patients and healthy controls to determine whether single nucleotide polymorphisms in the α2M-gene (exon 24, Val1000Ile), LRP-gene (exon 6, Ala216Val), RAP-gene (exon 7, Val311Met) predispose their carriers to AD.

Individuals were recruited in outpatient clinics for cognitive disorders from the Department of Psychiatry, Technical University Munich, and the Department of Neurology, Philipps University Marburg, Germany. The study consisted of 271 patients with AD (mean: 70.7 ± 9.6 years; range: 50–95 years; 37% woman) and 280 age-/gender-matched non-demented control subjects (mean: 66.9 ± 10.6 years; age range: 50–93 years; 31% women). All patients met ICD-10 criteria for dementia [24] as well as NINCDS-ADRDA criteria for probable or possible AD [14]. All normal subjects were documented to have no significant decline or impairment in cognition on clinical examination. All patients had given written informed consent.

Genomic DNA was extracted from blood according to standard protocols (Quiagen). Genotyping of *APOE* was performed as described previously [5]. Genotyping was performed with PCR-based amplification followed by restriction endonuclease digestions with *Sau*3AI for the G/A mutation at codon 1000 in α2M, *Ava*II for the C/T mutation at codon 216 in LRP and with *Nla*III for the G/A mutation at codon 311 in RAP. Endonucleases were purchased from New England Biolabs. Following primers were used: 5′-GGAGACATATTAGGCTCTG-3′ and 5′-CTGAAACCTACTGGAAATCC-3′ for α_2M; 5′-GCCTCTGCCACAGTGCTAACTA-3′ and 5′-ATACGGTCTCGTTGGCATAGCT-3′ for LRP; 5′-CTATGGAAACCTTGTTCCAGGA-3′ and 5′-ACTCGGACAGGGAAGACAACAG-3′ for RAP. Restriction fragments were separated on a 2%-agarose gel electrophoresis and visualized using SYBR gold staining on an UV-transilluminator. Analysis of odds ratios was conducted controlling for age group- and gender group-stratified Mantel–Haenszel odds ratios, CIs and hypothesis test. Statistical significance was considered at the level $p < 0.05$.

The *APOE*-ε4 allele frequency was higher in patients with AD than in control subjects (Table 1) confirming previous observations [15]. The genotype distributions for the investigated genes were in Hardy–Weinberg equilibrium for both AD and the control population. The allele and genotype frequencies of α2M, LRP and RAP polymorphisms in AD and controls individuals are displayed in Table 2. Inheritance of α2M conferred an increased risk to develop AD. The estimation of the Mantel–Haenszel odds ratio for being affected as a function of carrying at least one mutated α2M allele was 1.47 compared to no copy of the mutated α2M allele (0.56). We found no evidence of an interaction between the *APOE*-ε4 allele and mutated α2M with regard to the conferred risk. No genotype-specific effect of age on the distribution of the polymorphic α2M allele was found. Analysis for gender did not increase the magnitude of risk to develop AD for patients carrying the mutated α2M allele.

Table 1
Frequency of different *APOE*-ε alleles (as percentages) in the cohort of Alzheimer's diseased patients and controls

APOE	Ctrl			AD		
	/2	/3	/4	/2	/3	/4
ε2	0.4	15.0	2.6	0	6.8	2.0
ε3	15.0	55.6	24.1	6.8	47.1	36.0
ε4	2.6	24.1	2.3	2.0	36.0	8.1

/2, /3 or /4 are for carrying a second *APOE* allele ε2, ε3 or ε4, respectively. Ctrl, control subjects; AD, Alzheimer's diseased patients.

In our study population neither the allele frequency of the LRP polymorphism nor of the RAP polymorphism were significantly increased in patients with AD compared to controls (Table 2). The estimated odds ratios for being affected as a function of carrying one LRP-Val^{216} allele was 0.71 and 1.0 for carrying one RAP-Met^{311} allele, compared to carrying only wildtype alleles. No homocytic carriers of LRP-Val^{216} and RAP-Met^{311} alleles were observed in AD and control. No genotype-specific effect of age or gender was found in the distribution of the LRP allele or of the RAP allele. In addition, there is no interaction between *APOE*-ε4 and LRP or RAP.

It is suggested that the polymorphism in exon 24 of the α2M-gene, which causes an amino acid change at position 1000 of the α2M protein increases the risk to develop AD [12]. In that report the odds ratio for AD associated with the G/G genotype was 1.77 and in combination with *APOE*-ε4 9.68. Several studies have examined the α2M Val1000Ile with conflicting results [4,22]. We found a small increase in the risk to develop AD as a function of carrying at least one α_2M-Ile^{1000} allele with an odds ratio of 1.47. Although, our data confirm an association of α2M-Ile^{1000} with AD, there is only weak evidence as compared to the report of Liao et al. [12]. The Val1000Ile poly-

Table 2
Allele and genotype frequencies of α2M Val1000Ile, LRP Ala216Val and RAP Val311Met in Alzheimer's diseased patients and controls

Polymorphisms	Allele frequencies			Genotype frequencies		
	n	wt	mt	wt/wt	wt/mt	mt/mt
α_2M, Val1000Ile						
AD	271	0.323	0.677	0.089	0.469	0.443
Ctrl	280	0.430	0.570	0.161	0.539	0.300
LRP, Ala216Val						
AD	155	0.974	0.026	0.948	0.052	0.000
Ctrl	160	0.966	0.034	0.931	0.069	0.000
RAP, Val311Met						
AD	179	0.975	0.025	0.950	0.050	0.00
Ctrl	154	0.974	0.026	0.948	0.052	0.00

wt, wild type; mt, mutated type (polymorphism); α2M, α2-macroglobulin, LRP, low-density lipoprotein receptor-related protein; RAP, receptor-associated protein; AD, Alzheimer's diseased patients; Ctrl, control subjects; *n*, number of subjects.