Genetic Interaction between Tau and the Apolipoprotein E Receptor LRP1 Increases Alzheimer's Disease Risk

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Key Words

Alzheimer's disease • Tau phosphorylation • Low density lipoprotein receptor-related protein 1 (LRP1) • Cholesterol • Genetic polymorphism

Abstract

Abnormal tau hyperphosphorylation is one of the central events in the development of neurofibrillary tangles (NFTs) in Alzheimer's disease (AD), and phosphorylation of tau is accelerated by the increase in the level of neuronal cholesterol. Apolipoprotein E (APOE) promotes the neuronal uptake of cholesterol via APOE receptors such as the low-density lipoprotein receptor-related protein 1 (LRP1), and the APOE ϵ 4 allele is associated with an increase in NFT burden in AD brain. In a case-control study in 246 AD patients and 237 healthy controls, we examined whether the combined gene effects between tau (intron 9, rs2471738) polymorphism and LRP1 (exon 3, rs1799986) polymorphism might be responsible for susceptibility to AD, independently or in concert with the APOE ϵ 4 allele. Subjects carrying both the tau (intron 9, rs2471738) T allele (CT and TT genotypes) and the LRP1 (exon 3, rs1799986) T allele (CT and TT genotypes) had a 6 times higher risk of developing AD than subjects without these risk genotypes (odds ratio = 6.20, 95% confidence

interval = 1.74–22.05, $p = 0.005$), and this genetic interaction was observed in either the presence or the absence of the APOE ϵ 4 allele. These data suggest that the synergistic effects (epistasis) between tau and LRP1 might modify the risk of AD in an APOE ϵ 4 allele-independent fashion.

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Introduction

Abnormal tau hyperphosphorylation has been suggested as being one of the central events in the development of neurofibrillary tangles (NFTs), which are one of the characteristic neuropathological lesions found in Alzheimer's disease (AD) brains [1]. Defects in cholesterol metabolism could play a role in the abnormal phosphorylation of tau; in fact, mean levels of free cholesterol in tangle-bearing neurons are higher than those of adjacent tangle-free neurons in AD patients [2]. In addition, apolipoprotein E (APOE) promotes the release of glial cholesterol and the neuronal uptake of cholesterol via APOE receptors such as the low-density lipoprotein receptor-related protein 1 (LRP1) [3], and the APOE ϵ 4 allele, the main recognized genetic risk factor for sporadic AD, is associated with an earlier onset of NFT formation

and an increased NFT load [4, 5]. Moreover, accumulation of NFTs that are structurally and immunologically indistinguishable from those seen in AD are found in the brains of patients with Niemann-Pick type C disease, which is a cholesterol storage disease characterized by the accumulation of cholesterol in lysosomes [6, 7]. Genetic variability in LRP1 [8, 9] and tau [10] genes has been proposed to modify the risk of AD through an independent effect. The postulated common pathway of LRP1 and tau in the development of NFT prompted us to examine the combined contribution of these genes to the susceptibility for AD, independently or in concert with the APOE ϵ 4 allele. We focused on a functional polymorphism in the LRP1 (exon 3, rs1799986) gene and we also examined the tau (intron 9, rs2471738) genetic variant since this has been reported to alter the susceptibility to AD [10].

Patients and Methods

The study included 246 AD patients (65% women; mean age at the time of study 76.6 years; SD 7.3; range 62–97 years; mean age at onset 72.9 years; SD 7.1; range 60–93 years) who met NINCDS/ADRDA criteria for probable AD [11]. All AD cases were defined as sporadic because their family history did not mention any first-degree relative with dementia, this information being obtained by direct interview of relatives; patients with familial AD were excluded. AD patients were consecutively admitted to the Department of Neurology, University Hospital ‘Marqués de Valdecilla’, Santander, Spain, from January 1997 to December 2001. The large majority of patients lived in the community and had been referred by their general practitioner; few had been admitted from hospital wards or nursing home facilities. Control subjects were 237 unrelated individuals (69% women; mean age 81.2 years; SD 7.7; range 63–100 years) randomly selected from a nursing home. These subjects were evaluated by specialist neurologists, and for the diagnosis of a normal cognitive status, the following criteria had to be met: no active neurological or psychiatric disorder; a normal neurological exam; no psychotropic drugs; no ongoing medical problems interfering with cognitive function; living and functioning independently in the community; and cognitive performance assessed by means of Mini-Mental State Examination with scores ≥ 28 , which were verified by at least one subsequent annual follow-up assessment. The control subjects were significantly older than patients raising questions on age-mortality selection bias; however, the fact that control genotypes were in Hardy-Weinberg equilibrium and that there were no differences in the distribution of tau and LRP1 gene alleles according to the age of controls divided into quartiles argued against a significant bias being introduced by this method of selection. The controls arose from the same base population as the cases. The AD and control samples were Caucasians originating from a homogeneous population in a limited geographical area in Northern Spain. All patients and controls were ascertained to have parents and grandparents born in Northern Spain to ensure ethnicity. Consequently, possible confounding effects of

the inclusion in the study of members of different ethnic groups have been minimized.

Blood samples were taken after written informed consent had been obtained from the subjects or their representatives. The study was approved by the ethical committee of the University Hospital ‘Marqués de Valdecilla’. Genotyping of tau (intron 9, rs2471738) polymorphism was performed by a Taq-Man single-nucleotide polymorphism assay (Applied Biosystems, Warrington, UK) and an ABE PRISM 7000 sequence detection system (Applied Biosystems). The LRP1 (exon 3, rs1799986) polymorphism was determined as described previously [8].

Statistical Methods

Association between dichotomous variables was analyzed with odds ratio (OR), and 95% confidence intervals (CI) were estimated by the Cornfield method or the exact method. *p* values were estimated by χ^2 or Fisher exact tests. For the adjusted analyses, we generated a logistic regression model that includes as the independent variables the age at onset, gender, APOE (categorized into 2 groups corresponding to the presence or absence of the APOE 4 allele), LRP1 (exon 3, rs1799986) (grouped into 2 categories: CC and CT+TT genotypes) and tau (intron 9, rs2471738) (grouped into 2 categories: CC and CT+TT genotypes); the model also included interaction terms that were the product of the categories of LRP1 (exon 3, rs1799986) and tau (intron 9, rs2471738); the strength of the associations between LRP1 (exon 3, rs1799986) and tau (intron 9, rs2471738) genotypes and AD was summarized with OR and their 95% CI. All statistical analyses were performed with the package SPSS 13.0 for Windows (SPSS, Inc, Chicago, Ill., USA).

Results

Control groups for LRP1 (exon 3, rs1799986) ($p = 0.14$) and tau (intron 9, rs2471738) ($p = 0.81$) polymorphisms were within the range of the Hardy-Weinberg equilibrium. When the risk was considered for a single polymorphism, neither the presence of the LRP1 (exon 3, rs1799986) T allele (CT+TT genotypes) nor the presence of the tau (intron 9, rs2471738) T allele (CT+TT genotypes) was associated with AD (table 1); however, the subjects carrying both genotypes had a 6.20-fold increased risk of developing AD than subjects without these risk genotypes (adjusted by age, sex and APOE status: OR = 6.20, 95% CI = 1.74–22.05, $p = 0.005$), suggesting epistasis or gene-gene interaction (table 2); the interaction between the 2 loci remained significant after conservative multiple testing Bonferroni correction ($p = 0.015$). Our analysis showed the expected association between the APOE ϵ 4 allele and AD with an OR = 5.72 (95% CI = 3.81–8.57, $p < 0.0001$) for carrying 1 or 2 copies of ϵ 4 allele. Stratification of the data by APOE genotypes (table 2) indicated that the risk effect of the interaction of the LRP1 (exon 3, rs1799986) T allele and the tau (intron

Table 1. Distribution of tau intron 9 (rs2471738) and LRP1 exon 3 (rs1799986) polymorphisms in patients and controls stratified by APOE ϵ 4 allele

Poly-morphism	Total sample		APOE ϵ 4 allele non-carriers		APOE ϵ 4 allele carriers	
	patients	controls	patients	controls	patients	controls
Tau (intron 9)						
CC	173 (0.70)	170 (0.72)	71 (0.70)	132 (0.70)	102 (0.70)	38 (0.79)
CT	64 (0.26)	62 (0.26)	27 (0.27)	52 (0.27)	37 (0.26)	10 (0.21)
TT	9 (0.04)	5 (0.02)	3 (0.03)	5 (0.03)	6 (0.04)	0 (0.00)
Total	246	237	101	189	145	48
Allele frequency C/T	0.83/0.17	0.85/0.15	0.84/0.16	0.84/0.16	0.83/0.17	0.90/0.10
LRP1 (exon 3)						
CC	193 (0.78)	198 (0.83)	76 (0.75)	154 (0.81)	117 (0.81)	44 (0.92)
CT	51 (0.21)	35 (0.15)	25 (0.25)	32 (0.17)	26 (0.18)	3 (0.06)
TT	2 (0.01)	4 (0.02)	0 (0.00)	3 (0.02)	2 (0.01)	1 (0.02)
Total	246	237	101	189	145	48
Allele frequency C/T	0.89/0.11	0.91/0.09	0.88/0.12	0.90/0.10	0.90/0.10	0.95/0.05

Figures in parentheses indicate frequencies; $p > 0.05$ for all comparisons.

Table 2. Odds ratios for interaction between tau intron 9 (rs2471738) and LRP1 exon 3 (rs1799986) in the total sample and after stratification by APOE genotype

	Tau (intron 9) allele T (CT+TT genotypes)	LRP1 (exon 3) allele T (CT+TT genotypes)	AD cases	Controls	OR (95% CI)	p
Total sample	–	–	136	135	1 (reference)	
	+	–	57	58	0.97 (0.57–1.67)	0.936
	–	+	37	35	1.36 (0.71–2.58)	0.342
	+	+	16	4	6.20 (1.74–22.05)	0.005
APOE ϵ 4 (–)	–	–	55	100	1 (reference)	
	+	–	21	49	0.66 (0.34–1.28)	0.224
	–	+	16	32	0.92 (0.44–1.94)	0.842
	+	+	9	3	6.35 (1.47–27.27)	0.013
APOE ϵ 4 (+)	–	–	81	35	1 (reference)	
	+	–	36	9	2.74 (0.89–8.48)	0.079
	–	+	21	3	3.92 (0.77–19.83)	0.098
	+	+	7	1	11.90 (0.90–156.25)	0.059

Tau (intron 9) allele T (–) = CC genotype; LRP1 (exon 3) allele T (–) = CC genotype; APOE ϵ 4 (–) = no copies of ϵ 4; APOE ϵ 4 (+) = 1 or 2 copies of ϵ 4 allele.

ORs are adjusted by age, sex and APOE genotype (using multiple logistic regression) in the total sample, and adjusted by age and sex in the APOE ϵ 4 (–) and APOE ϵ 4 (+) subgroups.

9, rs2471738) T allele was present in the APOE ϵ 4 negative group ($p = 0.013$), while the interaction LRP1 and tau conferred a marginally significant increase in the risk in the APOE ϵ 4 carriers ($p = 0.059$), owing to the low number of subjects in the control group.

Discussion

We have shown, for the first time, that the LRP1 and tau genes may interact in determining the risk for AD, with subjects carrying both the LRP1 (exon 3, rs1799986)

T allele (CT+TT genotypes) and the tau (intron 9, rs2471738) T allele (CT+TT genotypes) having a 6.20-fold increase in AD risk. The discovery that APOE ϵ 4 allele is a risk factor for sporadic AD raised the possibility that the receptors to which APOE binds on the surface of neurons would also be involved in the neurodegenerative process [3]. Levels of LRP1, one of the major APOE receptors in the brain, are increased on neurons in AD hippocampus [12]. Analysis of LRP1 genotype status has revealed significantly higher LRP1 brain levels among subjects harboring the LRP1 (exon 3, rs1799986) CT or TT genotypes compared with carriers of the CC genotype [13]. This upregulation of LRP1 might result in accumulation of cholesterol in neurons of LRP1 (exon 3, rs1799986) T allele carriers. Alterations of intracellular cholesterol levels could influence hyperphosphorylation of tau and NFT formation in the brains of patients with AD; several reports have described a higher stage of NFTs in AD patients with an APOE ϵ 4 allele when compared to APOE ϵ 3 homozygotic AD individuals [4] and transgenic overexpression of human APOE4 in neurons increases tau phosphorylation in mice [5]. It is accepted that the ability of APOE4 to promote cholesterol uptake by neurons through the interaction with its APOE receptors, such as LRP1, seems to be greater than that of the other 2 major APOE isoforms, E2 and E3 [14]; consequently, it is possible that isoform-specific APOE might influence the free cholesterol levels of neurons, thereby mediating the APOE4 effect on NFT formation. Neurodegeneration in association with hyperphosphorylation of tau in NFTs has also been found in the brains of patients with Niemann-Pick type C disease [6, 7], a hereditary cholesterol disorder in childhood and adolescence characterized by the inability to process cellular cholesterol that accumulates within lysosomes. Although the mechanism by which cholesterol modulates NFT formation has not been established, fibroblasts from Niemann-Pick type C disease displayed enhanced expression of a number of kinases (tau tubulin kinase 1, mitogen-activated protein kinase 8, protein kinase C gamma) that may participate in tau phosphorylation [15].

Several conflicting studies have reported on an association between LRP1 (exon 3, rs1799986) and AD, some showing a positive association with the C allele, one showing a positive association with the T allele, while other studies showed no association with AD [8, 9]. Our previous findings had shown no independent association of LRP1 with AD [8] and the present study confirms these results, but indicates that an AD risk of LRP1 (exon 3, rs1799986) T allele is only apparent in the presence of the tau (intron 9, rs2471738) T allele. Our study exemplifies

that the interactive effect between polymorphisms of 2 genes (epistasis) may exist without a significant main effect of either; in such cases, the effect would have been missed if polymorphisms of both genes had not been tested jointly [16]. Several studies on variability within tau and the occurrence of AD have been published with inconclusive results, but the region of strongest genetic association in tau is between just upstream of exon 1 and just within intron 9 [10]; the genetic variability in this region could influence the expression levels of tau [17]. The tau (intron 9, rs2471738) T allele has been associated with an increased AD risk [10], but this variant does not appear to interrupt the splice site, suggesting that this variant is in linkage disequilibrium with other functional variants. Our sample size had 99% power to detect an OR of 6.2 for a risk effect of LRP1 (exon 3, rs1799986) T allele and tau (intron 9, rs2471738) T allele on AD risk. A limitation of our study is the sample size, which does not allow for a rigorous analysis of APOE-specific differences in AD risk associated with the synergistic effect of LRP1 and tau, as stratification of the sample resulted in rather small subsample of APOE ϵ 4 carrier subjects. However, our data suggest that the interaction between LRP1 and tau genes might modify the risk of AD in an APOE ϵ 4 allele-independent fashion, with the AD risk of the interaction being observed in either the presence or the absence of the APOE ϵ 4 allele. Although testing the interaction of the different haplotypes in tau and LRP1 genes deserves further attention, we focused on the interaction of only a single potentially functional polymorphism in each one of these genes. Our positive findings are based on a small number of cases and controls and therefore, additional studies are required to confirm the synergistic effects (epistasis) of LRP1 and tau on the risk of AD.

Evidence from in vitro and in vivo studies suggests that increased cellular cholesterol levels induce high amyloid beta production, which is central to the pathogenesis of AD [18]. The possibility to modulate the intraneuronal elevated cholesterol levels by cholesterol-lowering drugs (statins) has a substantial therapeutic implication for AD. In fact, treatment with statins has been associated in some epidemiological studies with a reduced risk of AD [19]; statins may slow cognitive decline in mild to moderate AD [20, 21] and statin therapy is associated with reduced NFT burden at autopsy in a population-based sample of human subjects [22] and in a transgenic mouse model of tauopathy [23]. However, recently reported prospective randomized studies have been negative for primary prevention and also failed to slow progression of disease in patients with established mild to moderate AD [24].

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