Influence of lysosomal acid lipase polymorphisms on chromosome 10 on the risk of Alzheimer’s disease and cholesterol metabolism

K.-T. von Trotha, R. Heun, S. Schmitz, D. Lütjohann, W. Maier, H. Kölsch

Department of Psychiatry, Sigmund-Freud Street 25, University of Bonn, 53105 Bonn, Germany

Received 17 February 2006; received in revised form 22 March 2006; accepted 10 April 2006

Abstract

Linkage analyses have identified a possible hot spot for a late-onset Alzheimer’s disease (LOAD) risk gene on chromosome 10q21-22 and 10q25. It was also shown that cholesterol metabolism is involved in the pathogenic mechanisms of AD. The gene of lysosomal acid lipase (LIPA) is located next to the putative hot spot on chromosome 10. Its protein is involved in cholesterol metabolism and responsible for catalysing the hydrolysis of cholesteryl esters and triglycerides inside the lysosome. Previous publications reported controversial results on the role of LIPA polymorphisms on the risk of LOAD. We investigated two LIPA polymorphisms (rs1051338 and rs2297472) for their putative effect on the risk of LOAD in a homogenous sample of German origin. Genotypes of the investigated polymorphisms in AD patients and controls were compared. Also the effect of the LIPA gene polymorphisms on plasma cholesterol levels and 24S-hydroxycholesterol/cholesterol ratios on AD patients were investigated. None of the observed polymorphisms showed a significant influence on the risk of AD. We found that LIPA exon 2 polymorphism (rs1051338) influenced plasma 24S-hydroxycholesterol/cholesterol ratios in AD patients where carriers of the C/C allele presented with higher ratios than heterozygote carriers of the LIPA allele. Even though the biological function and gene location of LIPA on chromosome 10 suggest that LIPA might be a candidate for an AD risk gene, our results revealed that polymorphisms in LIPA did not influence the risk of AD in our study.

© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: Lysosomal acid lipase (LIPA); Alzheimer’s disease; Chromosome 10; Polymorphism; Association

Alzheimer’s disease (AD) is a progressive, chronic neurodegenerative disease of the central nervous system and the leading cause of dementia worldwide. Neuropathological characteristics are senile plaques, vascular amyloid deposits, neurofibrillary tangles and increased neuronal cell death [3,14]. Environmental and genetic determinants influence the risk of AD. While mutations in amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2), have been shown to be causally linked to familial early-onset AD, until now, the apolipoprotein E4 (APOE4) allele is the only confirmed genetic risk factor for late-onset AD (LOAD) [31]. However, the APOE4 allele is neither sufficient nor necessary for the development of LOAD. Epidemiological studies revealed that additional genetic risk factors might contribute to the late-onset form of AD [12,15,20,24]. Recent observations indicate that cholesterol metabolism is involved in the pathogenesis of AD. Different studies have shown that cholesterol blood levels were altered in AD patients compared to controls [18,22,27]. Levels of plasma 24S-hydroxycholesterol, a metabolite of brain cholesterol, are significantly lower in patients with AD compared to healthy controls [7,19], and depletion of cholesterol inhibits the production of β-amyloid (Aβ) in vitro [30]. Cholesterol lowering 3β-hydroxy-3β-methyl-glutaryl-CoA reductase inhibitors, so-called statins, reduce the levels of Aβ protein in vitro and in vivo [10]. Statins might reduce the risk of AD [16,32], even though this pharmacological therapy for AD is also discussed controversially [21]. Full genome screens for AD found significant evidence for linkage on chromosomes 9, 10 and 12 [17,26]. In detail, the chromosomal regions of 10q21-22 and 10q25 have been linked to increased LOD scores for AD [5,6,25]. One gene located in this region is lysosomal acid lipase (LIPA) on chromosome 10q23.2-q23.3, consisting of 10 exons [2]. Lysine A catalyzes the hydrolysis of cholesteryl esters and triglycerides in the lysosome. Mutations in this gene cause Wolman disease and cholesteryl ester storage disease [1]. Polymorphisms in this gene have been investigated for their putative effect on the risk of AD; however, results are controversially discussed. While Riemenschneider et al. [29] found no influence of LIPA polymorphism...
on the risk of AD. Papassotiropoulos et al. [28] showed that LIPA polymorphism might contribute to the risk of AD.

Thus, we studied two polymorphisms in LIPA in a homogeneous German population of AD patients and controls on the risk of AD. Also the possible effect of those polymorphisms on plasma cholesterol and 24S-hydroxycholesterol in AD patients was investigated. The first polymorphism is a known A/C exchange located in exon 2 (rs1051338), causing an amino acid exchange from threonine to proline at amino acid position 16 of the protein. Threonine is a polar and hydrophilic amino acid, while proline is nonpolar and hydrophobic, thus this polymorphism might cause functional alterations of the enzyme. Functional studies have not been performed up to now but seem increasingly necessary. A second C/T polymorphism, which has not been verified in a human population before, is located in intron 5 five bp upstream of exon 6 (rs2297472) and this polymorphism might cause functional alterations of the amino acid, while proline is nonpolar and hydrophobic, thus this polymorphism might cause functional alterations of the enzyme.

Functional studies have not been performed up to now but seem increasingly necessary. A second C/T polymorphism, which has not been verified in a human population before, is located in intron 5 five bp upstream of exon 6 (rs2297472) and might influence the splice site. The functional relevance of both polymorphisms has not been investigated up to now.

LIPA polymorphisms were investigated in 244 AD patients (age = 73 ± 9.3 years, range = 48–96 years, 72.2% female), recruited from the Department of Psychiatry, University of Bonn. AD patients were diagnosed according to DSM-IV, supported by clinical examination, detailed structured interviews, neuropsychological testing, cognitive screening, including Mini-Mental State Examination scores and neuromaging studies [11]. Two hundred and eighty-five age-matched healthy controls (age = 72 ± 8.3 years, range = 61–100 years, 56.5% female) from the general population were recruited with the support of the local Census Bureau and the regional Board of Data Protection (North-Rheine-Westfalia, Germany). Cognitive status of healthy controls was assessed by neuropsychological testing and structured interviews. All participants of the study gave informed written consent. The study has been approved by the Ethics Committee of the Faculty of Medicine of the University of Bonn.

Genomic DNA was extracted from leukocytes using the Qia-gen blood isolation kit according to the instructions of the manufacturer (Qiagen, Hilden, Germany).

The A/C polymorphism (rs1051338) in exon 2 was analyzed by confronting two-primer polymerase chain reaction (CTPP-PCR) using the following oligonucleotides designed from Acc no. NT_030059: 5′-GAAGTAAAGTGCCTCTCTCTG-3′ (forward) and 5′-TGAGCCGGGAATGATGTCG-3′ (reverse). To generate allele specific amplification products, two additional allele specific primers 5′-CCTGGTGTTCCATGTAGTCT-3′ (forward, T-allele) and 5′-GTTAGAAATGCTCATGAAAAACCTGGT-3′ (reverse, A-allele) were included into the CTPP-PCR. The amplification products of the T and the C alleles were 227 and 178 bp or 227 and 95 bp. Samples were separated using a 2% agarose gel and visualized with the Gelstar® staining kit.

For analysis of cholesterol and 24S-hydroxycholesterol blood samples of 111 AD patients (age = 71.1 ± 9.105 years, 60.4% female) were collected into EDTA-containing tubes. Aliquots were centrifuged at 2000 × g for 10 min and the supernatant was stored at −20 °C until analysis. Plasma concentrations of cholesterol were measured by standard enzymatic procedures (Boehringer, Mannheim, Germany). Total plasma concentrations of 24S-hydroxycholesterol were measured by a modified highly sensitive method using combined gas chromatography/mass spectrometry as described previously [9,23].

The effect of LIPA exon 2 and exon 6 polymorphisms on plasma cholesterol levels and on the ratio of plasma 24S-hydroxycholesterol/cholesterol were tested by MANOVA including the APOE4 allele, sex and age as covariates. Genotype distribution of both polymorphisms in AD patients and controls were compared by Pearson's two-sided $p$-test. Logistic regression analysis investigated the simultaneous effect of LIPA exon 2 A/C polymorphism and exon 6 C/T polymorphism, APOE4 allele, sex and age on the risk of AD. For LIPA exon 2 A/C polymorphism the statistical power to detect an odds ratio (OR) = 2.0 in carriers of a C-allele with $\alpha = 0.05$ was power = 97% in AD patients, taking the observed allele frequencies in controls (C-allele, 0.28; A-allele, 0.72) as a reference. The statistical power to detect an odds ratio (OR) = 2.0 for LIPA exon 6 C/T polymorphism in carriers of a T-allele with $\alpha = 0.05$ was power = 97% in AD patients, taking the observed allele frequencies in controls (T-allele, 0.22; C-allele, 0.78) as a reference. Statistical significance was established at $\alpha = 0.05$. Statistic tests were carried out using SPSS (10.0).

The Hardy–Weinberg equilibrium could be verified for the tested population. There was no significant difference of the genotype distribution of LIPA exon 2 or exon 6 polymorphisms between AD patients and healthy subjects (Table 1).

MANOVA revealed, that AD patients who were carriers of the LIPA exon 2 C/C genotype showed significantly higher plasma 24S-hydroxycholesterol/cholesterol ratios than carriers of the A-allele, whereas the LIPA exon 6 polymorphism did not show an influence on 24S-hydroxycholesterol/cholesterol ratios (for details see Table 2). The covariates APOE4 allele, sex and age did not influence plasma 24S-hydroxycholesterol/cholesterol ratios in this model (APOE4: $F = 1.245, d.f. = 1, p = 0.273$; sex: $F = 0.574, d.f. = 1, p = 0.450$; age: $F = 1.214, d.f. = 1, p = 0.273$).

Plasma cholesterol levels were not influenced by any of the investigated LIPA polymorphisms (Table 2). We did not also find an influence of the covariates APOE4 allele, sex and age on plasma cholesterol in this model (APOE4: $F = 0.381, d.f. = 1, p = 0.538$; sex: $F = 2.260, d.f. = 1, p = 0.136$; age: $F = 2.081, d.f. = 1, p = 0.152$).

Logistic regression analysis revealed no significant influence of the LIPA polymorphism in exon 2 ($\chi^2 = 0.118, d.f. = 1, p = 0.731$) or in exon 6 ($\chi^2 = 1.048, d.f. = 1, p = 0.306$) on the
The risk for LOAD was observed (risk of LOAD. Also no interaction of both polymorphisms on AD patients 245 0.79 0.21 159(64.9) 70 (28.6) 16 (6.5)

Controls 285 0.78 0.22 181(63.5) 84 (29.5) 20 (7.0) 0.123 2 0.941

Exon 6 C/T

AD patients 245 0.71 0.29 122(49.2) 104 (42.4) 19 (7.8)

Controls 285 0.72 0.28 143(50.2) 126 (44.2) 16 (5.6) 1.013 2 0.603

Table 2

<table>
<thead>
<tr>
<th>Exon 2 A/C</th>
<th>n</th>
<th>Allele frequencies</th>
<th>Genotypes</th>
<th>Genotypes</th>
<th>( \chi^2 )-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>C</td>
<td>CC (%)</td>
<td>AA (%)</td>
</tr>
<tr>
<td>Controls</td>
<td>285</td>
<td>0.72</td>
<td>0.28</td>
<td>143(50.2)</td>
<td>126 (44.2)</td>
</tr>
<tr>
<td>AD patients</td>
<td>245</td>
<td>0.71</td>
<td>0.29</td>
<td>122(49.2)</td>
<td>104 (42.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exon 6 C/T</th>
<th>n</th>
<th>Allele frequencies</th>
<th>Genotypes</th>
<th>Genotypes</th>
<th>( \chi^2 )-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>T</td>
<td>CC (%)</td>
<td>CT (%)</td>
</tr>
<tr>
<td>Controls</td>
<td>285</td>
<td>0.78</td>
<td>0.22</td>
<td>181(63.5)</td>
<td>84 (29.5)</td>
</tr>
<tr>
<td>AD patients</td>
<td>245</td>
<td>0.79</td>
<td>0.21</td>
<td>159(64.9)</td>
<td>70 (28.6)</td>
</tr>
</tbody>
</table>

Unexpectedly, we did not find a significant influence of the APOE4 allele on plasma levels of cholesterol in AD patients. Without a doubt, if is clear that the APOE4 allele influences plasma cholesterol in non-demented probands, in that carriers of the APOE4 allele present with increased plasma cholesterol levels. However, also previous studies described, that this effect might not be detected in AD patients [8]. The causes of these findings are not entirely clear. But it might be speculated, that such probands, who are carrier of an APOE4 allele also present with increased cholesterol levels and might develop other disorders but AD, such as cardiovascular diseases and atherosclerosis. (2) Subjects with AD who lack APOE4 may have a cluster of other risk factors that affect cholesterol levels. (3) Increased plasma cholesterol might be part of a metabolic process taking place in all AD subjects independent of genetic or other risk factors. Further studies are needed to verify these possibilities.

We found no effect of LIPA polymorphisms on the risk of LOAD. Also haplotype analysis using FAMHAP (Ver. 15. [4]) including both LIPA polymorphisms, did not find an influence of LIPA on the risk of AD (data not shown). Riemschneider et al. investigated two functional polymorphisms in LIPA and observed no association with AD [29]. Papassotiriopoulos et al. [28] investigated the role of LIPA polymorphisms within a large analysis including 12 cholesterol-related single nucleotide polymorphisms in different genes and 48 control polymorphisms [26]. Here, a contribution to cluster significance of less than 5% was found for one polymorphism located in the 3′-UTR region of LIPA only in interaction with other polymorphisms. In com-

Table 1

Influence of LIPA polymorphisms on plasma cholesterol levels and 24S-hydroxycholesterol/cholesterol ratios in AD patients

<table>
<thead>
<tr>
<th>LIPA exon 2 (rs1051338)</th>
<th>Genotypes</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>AA + AC</td>
</tr>
<tr>
<td>Cholesterol (mg/dl) (mean ± S.E.)</td>
<td>219.35 ± 21.39</td>
<td>226.29 ± 11.02</td>
</tr>
<tr>
<td>24S-hydroxycholesterol/cholesterol (ng/mg) (mean ± S.E.)</td>
<td>45.43 ± 5.05</td>
<td>33.32 ± 2.60</td>
</tr>
</tbody>
</table>

LIPA exon 6 (rs2297472)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \chi^2 )</td>
</tr>
<tr>
<td>Cholesterol (mg/dl) (mean ± S.E.)</td>
<td>214.33 ± 23.43</td>
</tr>
<tr>
<td>24S-hydroxycholesterol/cholesterol (ng/mg) (mean ± S.E.)</td>
<td>35.11 ± 5.53</td>
</tr>
</tbody>
</table>

S.E. = standard error.
parision to this APOE4 showed a relative contribution to the risk of AD of more than 40%.

Our study, as well as the study by Riemschneider et al. [27], did not find an effect of LIPA polymorphisms on the risk of AD, even though the location of this gene within a putative spot for AD risk-gene might suggest an influence on AD. While our study was adequately powered to detect an association with an OR of 2, we cannot exclude the possibility that there is only a weak influence of LIPA polymorphisms on the risk of LOAD. Since Pappasostopoulos only found a marginal effect on the risk of AD for one polymorphism in LIPA we conclude that LIPA is unlikely to act as a major risk factor for LOAD.

While the studied polymorphisms did not show an effect on the risk of AD, we found the LIPA exon 2 A/C polymorphism to influence brain cholesterol metabolism. As cholesterol metabolism plays an important role in the pathogenesis of LOAD, further studies are required to investigate the effect of LIPA polymorphisms on brain cholesterol metabolisms, especially on 24S-hydroxycholesterol.

Acknowledgement

This study is part of the German Dementia Competence Network and was funded by the German Federal Ministry for Education and Research (grant: 01GI0422).

References


