The Cathepsin D (224C/T) Polymorphism Confers an Increased Risk to Develop Alzheimer’s Disease in Men

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The lysosomal protease cathepsin D is likely involved in β-amyloidogenesis in Alzheimer’s disease (AD). There is evidence for a single nucleotide polymorphism (rs17571) of the cathepsin D gene to be associated with increased AD risk. However, little is known about gender-specific differences. Therefore, we performed a genetic association study focusing on gender-specific differences in 434 participants (219 AD and 215 controls). Screening of the rs17571 shows a significantly higher proportion of T-allele carriers among male Alzheimer patients (28.5%) when compared with male controls (13.8%, p = .013, pcorr = .039). The odds ratio was 2.48 (95% confidence interval: 1.14–5.58). There was no significant difference in the T-allele distribution in women. Including APOE4 status and age did not have an additional effect on the morbidity risk. Thus, our results support the idea that rs17571 confers an increased risk for AD in men but not in women. Further investigation should substantiate the role of gender for AD risk of rs17571.

Key Words: Cathepsin—Genetics—Dementia—Gender—Risk factor.

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In Alzheimer’s disease (AD), the central pathogenetic events are the derivation of β-amyloid (βA4) from the amyloid precursor protein (APP) and neurofibrillary tangle formation (1). Conglomeration of βA4 leads to deposition of one neuropathologic hallmark in AD: the amyloidogenic plaque. The processing of APP to βA4 is localized mainly in the endosomal–lysosomal system (2).

Cathepsin D (catD), an acid aspartic endopeptidase, is the major lysosomal constituent in humans (3,4). There is evidence that catD is involved in AD pathogenesis. catD has been found within neuritic plaques in AD brains (5,6). The content of catD in plaques depends on the amount of βA4 (7). Furthermore, messenger RNA expression of catD is increased in brains of AD patients (8). A single nucleotide polymorphism (SNP) in the catD gene (C224T, exon2, and rs17571), leading to an amino acid exchange from alanine to valine, was found to be associated with increased AD risk compared with healthy controls (odds ratio [OR]: 2.1, 95% confidence interval [CI]: 1.4–4.2, p < .001) in German Caucasians (9). This observation has been replicated in an independent study (OR: 3.1, 95% CI: 1.6–6.2, p < .001) (10). rs17571 is supposed to alter mature catD enzyme function in that it increases proenzyme secretion in vitro of catD in breast cancer cells (11,12). This led to the hypothesis that rs17571 could contribute to augmented βA4 deposition in AD (9).

Up to now, however, 21 replication studies could not confirm this positive association (13–32), either suggesting that rs17571 is a false-positive result or that the marker plays a minor role in the etiology of AD, ie, confers a smaller genetic risk than originally described (33). A third possibility, however, is a potential interaction of rs17571 with gender, which was investigated in 8 of 23 genetic association studies so far. Two of them reported that the interaction of rs17571 and APOE4 increases the risk on AD for men but not for women (27,30), whereas six studies failed to detect any gender-related effect on AD risk (9,10,19,20,25,31). The remaining 15 studies did not consider gender for their analyses.

Therefore, we examined whether the SNP rs17571 influences the risk to develop AD focusing on a potential specific role of gender. In addition, we explored the impact of APOE4 genotype status (34) and age on the association between cathepsin D genotype at rs17571 and AD to check for effect modification or confounding.

Materials and Methods

Sample

We recruited 219 AD patients (mean age 69.7 years, SD ± 10.0, 53% females) from the Memory Clinic of the Psychiatric Department of the University of Munich. Most patients were of self-reported European ancestry. The diagnosis of
AD was performed according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (35). In order to exclude forms of dementia other than AD, medical and family history, general medical and neurological examination, blood and cerebrospinal fluid (CSF) tests, and computer tomography scans were performed. The control group included 215 neurological and psychiatric healthy, age- and gender-matched participants (mean age 67.5 years, SD ± 11.8, 56% females) with similar geographical and ethnic background, who were also recruited at the Memory Clinic in Munich. All patients and controls gave informed consent to participate in the study, with procedural approval from the local ethic committee (Munich).

Genotyping

Leukocyte DNA was isolated using standard protocols. The Exon 2 of the catD gene was amplified by polymerase chain reaction using oligonucleotide primers (forward: 5′-GTG ACA GGC AGG AGT TTG GT-3′ and reverse: 5′-GGG CTA AGA CCT CAT ACT CAC G-3′). Each amplification reaction contained 1 μL DNA, 1 μL of each primer (0.4 pmol/μL; MWG Biotech, Ebersberg, Germany), 2.5 μL of a dNTP mixture (Invitrogen, Paisley, UK), 14.4 μL ddH₂O, 5 μL Buffer F (Buffer-kit; Invitrogen, Paisley, UK), and 0.1 μL (1.25 U) of taq polymerase (Boehringer Mannheim, Mannheim, Germany) in a final volume of 25 μL. After initial denaturation for 5 minutes at 94°C, the amplification reaction with 30 cycles started with 0.5 minute at 94°C, 0.5 minute at 63°C, and 1 minute at 72°C, followed by a final extension period of 5 minutes at 72°C. Each reaction contained three negative controls in order to detect possible contamination. The 317-bp long amplification product was digested overnight by the restriction enzyme MWOI (New England Biolabs, Beverly, MA) according to the manufacturer’s instructions, and the DNA fragments were electrophoresed on a 2% agarose gel. The rs17571 C-allele yielded two distinctive fragments of 168 and 82 bp, whereas the T allele was identified by a distinctive fragment of 250 bp (Figure 1A and B).

APOE4 genotypes were determined by the restriction enzyme approach using standard protocol procedure as described by Zivelin and colleagues (36).

Statistical Analysis

Using the data of Papassotiropoulos and colleagues (10), we did power calculations using QUANTO Version 1.2.3 (http://hydra.usc.edu/gxe). Two hundred (100) case–control pairs were estimated to yield a comparison-wise power of about 0.98 (0.80) to detect a dominant OR of 3 (α = 0.05; two-sided) assuming a minor allele frequency of 0.05. Thus, the study was well powered to replicate the initial finding. All genotype distributions of controls in the primary analyses were tested for Hardy–Weinberg equilibrium and no evidence for such deviations was detected (all exact two-sided p values > .2). With regard to the described situation, we analyzed our data for two primary confirmatory research questions and/or hypotheses: (a) whether there is any significant difference in the distribution of the rs17571 genotypes (dominant genetic model) among AD cases and controls in the whole sample and (b) whether there is any significant difference in the distribution of the rs17571 genotypes (dominant genetic model) among AD cases and controls in the subsamples of males and females. Each of the two hypotheses was tested using Fisher’s exact test for the 2 × 2 table under a dominant genetic model for the presence of a rs17571 T allele. Control of the family-wise Type I error control was done by the method of Bonferroni applying a significance level of α = 0.05/3 ≈ 0.016 (two-sided).

In addition, we explored the conferred risk of the rs17571 genotype if the apolipoprotein E e4 (APOE4) status, age,
Table 1. Differences in the Distributions of rs17571 Genotypes and Allele Frequencies in the Total and in the Gender-Stratified Subsamples

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype Counts (frequencies) n (%)</th>
<th>Allele Frequencies</th>
<th>Dominant Genetic Model*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C/C</td>
<td>C/T</td>
<td>T/T</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD cases</td>
<td>174 (79.5%)</td>
<td>43 (19.6%)</td>
<td>2 (0.9%)</td>
</tr>
<tr>
<td>Controls</td>
<td>176 (81.9%)</td>
<td>39 (18.1%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD cases</td>
<td>73 (71.6%)</td>
<td>27 (26.5%)</td>
<td>2 (2.0%)</td>
</tr>
<tr>
<td>Controls</td>
<td>81 (86.2%)</td>
<td>13 (13.8%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD cases</td>
<td>101 (86.3%)</td>
<td>16 (13.7%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Controls</td>
<td>95 (78.5%)</td>
<td>26 (21.5%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

Notes: AD = Alzheimer’s disease; CI = confidence interval; OR = odds ratio.
*Exact ORs and exact uncorrected two-sided p values (Fisher’s exact test).

RESULTS

The distribution of the rs17571 genotypes (and allele frequencies) in the total sample showed no significant difference between AD cases and controls (see Table 1) and the estimated uncorrected OR for rs17571 T-allele carriers and AD risk was 1.17 (95% CI: 0.70–1.94, p = .546). In order to detect possible interactions between gender and rs17571 genotype status, we stratified the sample according to gender. rs17571 shows a significantly higher proportion of T-allele carriers among male Alzheimer patients (28.5%) when compared with male controls (13.8%, p = .013, χ²corr = .039), and rs17571 T-allele status significantly increased the odds to develop AD in men even after correction for multiple testing (OR: 2.48, 95% CI: 1.14–5.58; see Table 1). In contrast, no such significantly increased risk was detected in women.

With regard to investigate the conferred AD risk of rs17571 genotype, we then investigated possible dependencies between APOE4 and rs17571 genotypes by stratifying the sample according to APOE4 carrier status. Among APOE4 noncarriers, the T allele was present in 15.4% AD cases and 18.6% controls (OR under the dominant genetic model: 0.80, 95% CI: 0.39–1.58, p = .52). Among APOE4 carriers, the T allele was present in 26.5% AD cases and 15.4% controls (OR under the dominant genetic model: 1.98, 95% CI: 0.78–5.47, p = .16). Thus, there was no strong evidence for a dependency between APOE4 and rs17571 risk allele status. Ignoring the rs17571 T-allele status, APOE4 status was significantly related to AD risk in our sample, which is in accordance with previous reports in Caucasians (34,37). We detected 102 APOE4-positive AD cases (46.6%) and 52 APOE4-positive controls (25.0%) as opposed to 117 APOE4-negative AD cases (53.4%) and 156 APOE4-negative controls (75%); OR under the dominant genetic model: 2.62, 95% CI: 1.70–4.04, p = 3.17 × 10⁻⁶).

Furthermore, as described in the Statistical Analysis section, we performed binary logistic regression analyses to estimate rs17571 AD ORs corrected for the covariates age, gender, and APOE4 status (Model 1). A second model also included the respective two-way interactions of age and gender with rs17571. In sum, both the effects of APOE4 for all individuals and the interaction of gender and rs17571 status in males indicated increased AD risk (see Table 2), which underlines the observation already described in Table 1. Similar to the analyses in the total sample, we also assessed the joint impact of rs17571, age, and APOE4 on AD risk (Model 1) with and without rs17571 × Age interaction in men only (see Table 3). Both rs17571 and APOE4 conferred increased AD risk in men; in women, only the effect of APOE4 was observable (data not shown). Including “Age × rs17571” for the male probands did not improve the model.

DISCUSSION

In accordance with previous reports (13–32), we failed to detect significant differences of genotype distribution and allele frequency of rs17571 in a German sample of AD cases and healthy controls, although this association has been reported previously (9,10). Our calculated OR (dominant genetic model) for rs17571 in the whole sample was 1.17 (95% CI: 0.70–1.94, p = .546). This is in agreement with the result of a recent meta-analysis of the “AlzGene database” (OR: 1.14, 95% CI: 0.98–1.33) (33).

Interestingly, however, the risk to develop AD was significantly increased in male rs17571 T-allele carriers (OR: 2.48, 95% CI: 1.14–5.58, p = .013). But we observed no effect for females (OR: 0.58, 95% CI: 0.27–1.20, p = .128; see Table 1). When modeling the ORs for rs17571 status in the total sample including an interaction of gender and rs17571, this analysis also indicated the increased AD risk (see Table 2).
Two studies report minor evidence for rs17571 conferred AD risk in men (27,30). Menzer and colleagues (27) conducted an association study on rs17571 with 324 Caucasian AD patients and 302 controls. In contrast to our findings, the OR for rs17571 in males was not significantly increased, although it was higher in males than in females (males [OR: 2.28, 95% CI: 0.84–6.17] and females [OR: 1.13, 95% CI: 0.59–2.16]). However, the authors report a significant rs17571 conferred risk for male AD cases compared with male control participants, after stratification of the sample according to APOE4 status. This was not the case for female AD cases when compared with female controls. The OR for male rs17571 T-allele positives in APOE4+ positives was 10.0 (95% CI: 1.22–81.91, p = .02; females [OR: 0.86, 95% CI: 0.32–2.33]). In accordance with Menzer and colleagues (27), Jhoo and colleagues (30) report that the rs17571 conferred risk on AD was higher in males than in females and “that the interaction of rs17571 and APOE4 was shown only in males” (30) in a sample of Korean Asians comprising 107 AD patients and 216 cognitively normal controls. In contrast to these findings, adding an interaction term for rs17571 and APOE4 to our Model 1 (Table 3) for the males resulted in an OR of 7.64 (95% CI: 0.78–74.41, p = .08) for the interaction. In any case, to substantiate such a relationship, much larger or pooled samples need to be assessed which is obvious when looking at the width of respective 95% CIs.

In a joint analysis of two independent association studies (10), it was calculated that carriers of both, rs17571 T allele and APOE 4 allele, had a pooled OR of 10 (95% CI: 5.1–19.6), which was five times higher than for rs17571 alone (9). The authors argue that this observation could indicate a possible link for the synergistic action of APOE4 and rs17571 in AD pathogenesis (10). However, we were not able to substantiate their results. Instead, our total data more likely indicate no increased risk for rs17571 status in the gender-combined analysis and that also APOE4 status did modify this observed effect. In males, however, we observed some evidence for the idea that both APOE4 and rs17571 status confer an independent risk for AD.

Discrepancies in study findings might partly be explained by different genotype frequency depending on ethnicity. The genotype frequency for rs17571 in AD and controls in the whole sample of the present study regarding C/C was 80.6% in AD cases and 81.0% in controls. The C/T genotype was present in 18.3% AD cases and in 19.0% of the controls. The T/T genotype was present in 2.0% of the AD cases and in 0.0% of the controls. Our results of rs17571 genotype frequency in German Caucasians are in accordance with findings of the study by Menzer and colleagues (27) and with the meta-analysis of the AlzGene database (33). Interestingly, pooled genotype frequency of three studies with Asian ethnicity in the AlzGene database revealed that the T allele was less frequent in Asians than in Caucasian populations (33). The genotype frequency regarding the C/C genotype was 95.4% for AD cases and 95.8% for controls. The C/T genotype was present in 4.6% of the AD cases and in 4.1% of the controls. The T/T genotype was present in 0.0% of AD cases and in 1.0% of controls. This is also in accordance with the genotype frequencies reported by Jhoo and colleagues (30).

Another problem arises if a total sample is subdivided into subgroups (eg, by gender). Besides the obvious diminution of the sample size, this may also contribute to false-positive reports especially if subgrouping is done without any prior evidence (38). We cannot exclude the possibility that our results reflect chance findings even though a correction for multiple testing with strong control of the family-wise error rate was applied. Maximizing the study power by investigating much larger samples in cooperations (39) with a prespecified research questions, ie, focusing on specific Marker × Marker interactions, will reduce the probability of producing false-positive findings.

If our finding is not a false-positive report, it still remains unclear whether the increased risk of rs17571 in males reflects pathophysiological particularities peculiar to male

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**Table 2. Total Sample: Binary Logistic Regression Analyses**

<table>
<thead>
<tr>
<th>Predictor and Covariables</th>
<th>Model 1 OR (95% CI)</th>
<th>p Value</th>
<th>Model 2 (with interactions) OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17571 T allele−</td>
<td>1.09 (0.66–1.80)</td>
<td>.06</td>
<td>0.06 (0.01–3.13)</td>
<td>.16</td>
</tr>
<tr>
<td>APOE4</td>
<td>1.10 (1.01–1.21)</td>
<td>.04</td>
<td>1.09 (0.98–1.20)</td>
<td>.10</td>
</tr>
</tbody>
</table>

**Table 3. Male Probands: Binary Logistic Regression Analyses**

<table>
<thead>
<tr>
<th>Predictor and Covariables</th>
<th>Model 1 OR (95% CI)</th>
<th>p Value</th>
<th>Model 2 (with interactions) OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17571 T allele−</td>
<td>1.09 (0.98–1.20)</td>
<td>.02</td>
<td>1.09 (0.66–1.80)</td>
<td>.16</td>
</tr>
<tr>
<td>APOE4</td>
<td>2.37 (1.12–5.03)</td>
<td>.07</td>
<td>2.37 (1.12–5.03)</td>
<td>.07</td>
</tr>
</tbody>
</table>

**Note:** OR point estimates, 95% CIs, and p values (two-sided) from the Wald tests are reported; Model 1 is the multiple model with all main effects included only, whereas Model 2 is the multiple regression with all main effects and interactions included. CI = confidence interval; OR = odds ratio.
carriers. So far, no study has been published dealing with gender-specific differences regarding lysosomes or its components and the characteristic lesions in AD, the senile plaques. Moreover, there is no reproducible biological evidence that rs17571 influences AD pathogenesis, eg, plaque formation. Regarding the effects of rs17571 on CSF concentrations of tau protein and βA4, a study with two independent samples of AD patients (Germany \(n = 73\), a subsample of those reported here, and Sweden \(n = 66\)) demonstrated that presence of rs17571 T allele significantly decreased tau concentration in CSF, whereas no effect was detectable regarding βA4 concentration, suggesting that rs17571 might be associated with tau degradation rather than amyloid processing (40). Due to power concerns, gender-specific effects were not investigated but are a likely subject of future research in larger samples.

In sum, we are, to our knowledge, the first to report that the cathepsin D (C224T) polymorphism significantly increases the risk to develop AD in men only. Our findings support the hypothesis that rs17571 might act as a gender-specific risk factor. There are inconsistencies in study design, especially with regards to gender as a variable. Thus, our work will hopefully encourage researchers to reanalyze the available rs17571 data with regard to gender differences in a concerted and methodologically sound (38) action.

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