Cystatin C as a risk factor for Alzheimer disease

Abstract—Cystatin C, a protease inhibitor with widespread distribution, is upregulated in response to injury. Levels are elevated in the brains of patients with Alzheimer disease (AD). We compared frequencies for the CST 3 exon 1 polymorphism in patients with AD and controls. A proportional odds model indicated that the CST 3 A and APOE4 combination carried a high risk: a 14-fold elevation for men and 16-fold for women. These risks apply to risk at ages older than 64 years and to a shift in onset to ages younger than 65 years.

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Cystatin C is a proteinase inhibitor of the cathepsins. It has wide distribution and is localized to both neurons and glia. Expression increases in response to injury. The protein colocalizes with the Aβ peptide in brain amyloid deposits in patients with Alzheimer disease (AD), in senile plaque, and in vessel walls. At least four polymorphisms have been described: two in the 5′-untranslated region and in exons 1 and 2.

The gene for cystatin C is situated on chromosome 20, 20p11.2. It is 7.3 kb long and consists of three exons. A polymorphism associated with AD is located in exon 1: A G/A transition results in Ala/Thr as the penultimate amino acid of the signal peptide, thought to reduce secretion and constitutive extracellular levels. The GG genotype doubles the risk in patients at ages 80 and older. Subsequent studies did not replicate this finding or the associated risk with the A allele and at younger ages.

We compared the frequencies of CST 3 A and G alleles found for 179 AD cases and 141 spouse control subjects. The combination of one or two A alleles, i.e., the AG or AA genotypes, and APOE4 carried a high risk, shifting the onset to younger ages.

Methods. Subjects. There were 179 patients with AD (white; 126 female, 53 male; average age at onset 71 ± 8 years, 142 older than 65 years) and 141 spouse control subjects (white; 83 female, 58 male; average age at onset 72 ± 8 years, 120 older than 65 years). The clinical diagnosis of probable AD was made according to National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association criteria. Mean ages and sex frequencies for each case group were similar. The three groups were analyzed separately for population stratification by allele frequencies (see reference E-2), and no significant differences were found; thus, they were combined for the analysis.

Genotyping. APOE was genotyped as before (see reference E-3). The primers for the CST 3 exon 1 polymorphism and protocol were as described. The G/A transition resulted in the loss of a SstII restriction site, yielding an undigested 500-bp band (the AA genotype); bands at 357 and 143 bp indicated the GG genotype. Ten percent were genotyped in duplicate with consistent results.

Statistical analysis. Frequencies of alleles and genotypes found for case and control subjects were compared using Fisher’s exact or χ² test. Odds ratios and confidence limits were estimated (Statistica, version 6.1; StatSoft, Tulsa, OK). Estimated power calculations were also performed (see reference E-4).

A proportional odds model was constructed that considered three outcomes: 1 = onset before age 65, 2 = onset after age 65, 3 = age 65+ and unaffected (SAS Version 8.2 procedure LÓGISTIC with the proportional odds model option). Unaffected subjects younger than age 65 (n = 21) were excluded. The referent group was men lacking CST 3 A and APOE4. Risk was estimated in relation to sex, CST3 A, and APOE4, i.e., seven risk categories.

Results. The CST 3 A allele was more common for the patients with AD compared with the spouse control subjects (25% vs 17%; p = 0.02) (table 1), a 1.6-fold increment in risk of AA or AG genotypes (p = 0.06; 95% CI 1.0 to 2.5). There was threefold elevation in risk for APOE4 carriers who carried AA or AG (p = 0.0181; 95% CI 1.2 to 6.1) (table 2). CST 3 A was not a risk factor for subjects lacking the APOE4 allele, i.e., as ε24, ε34, or ε44 genotype.

There was some evidence that CST 3 A posed a higher risk for women: Women had a twofold increment, and APOE4-positive women had a fourfold increment (see table 2). There was also some evidence that risk pertained to ages at onset younger than 65 demonstrating a fivefold increased risk.

Taking these results into account, a proportional odds model was constructed with three outcomes: onset before age 65, onset after age 65, and unaffected at ages 65 and older (table 3). The predictors were combinations of the genetic risk factors CST3 A and APOE4 for men and women, i.e., a total of eight categories. Men without either genetic risk factor were the referent category whose relative risk was 1. We found that CST 3 A alone was not a risk factor: Relative risks for men and women who carried AA or AG and who did not carry APOE4 were close to the reference risk.
Table 1 CST 3 genotypes

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>17 (9.5)</td>
<td>6 (4.3)</td>
</tr>
<tr>
<td>AG</td>
<td>56 (31.3)</td>
<td>37 (26.2)</td>
</tr>
<tr>
<td>GG</td>
<td>106 (59.2)</td>
<td>98 (69.5)</td>
</tr>
<tr>
<td>A</td>
<td>90 (25.1)*</td>
<td>49 (17.4)*</td>
</tr>
<tr>
<td>G</td>
<td>268 (74.9)*</td>
<td>233 (82.6)*</td>
</tr>
<tr>
<td>A–</td>
<td>73 (40.8)*</td>
<td>43 (30.5)*</td>
</tr>
<tr>
<td>GG</td>
<td>106 (59.2)*</td>
<td>98 (69.5)*</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

† p = 0.0180; OR = 1.6; 95% CI = 1.1–2.4; standard power = 0.9227.
‡ p = 0.0574; OR = 1.6; 95% CI = 1.0–2.5; standard power = 0.7716.

The association speaks for itself and suggests different pathologic or selective mechanisms as an explanation of the association of CST 3 GG with AD among the oldest old. The lower risk found for women without either risk factor compared with men (odds ratio = 0.7, 95% CI = 0.5 to 1.0) suggests that this risk combination may partly account for the higher risk of AD generally reported for women and earlier AD brain changes.

Biologically, the increased expression of cystatin C in response to injury, high levels found in AD brains, and lower constitutive levels found for the risk A allele, i.e., altered signal peptide, make it a good candidate gene. Analytical data and consideration of APOE4, age at onset, and sex with CST3A as a risk factor compared with men (odds ratio = 0.7, 95% CI = 0.5 to 1.0) suggests that this risk combination may partly account for the higher risk of AD generally reported for women and earlier AD brain changes.

Acknowledgments
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References

**Ventromedial frontal lobe trauma**

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A 33-year-old man attempted suicide with a crossbow (figure), injuring his left ventromedial prefrontal cortex (VMPFC). He had a prior history of pathologic aggression and violent behavior. Afterward, he was docile, indifferent to his situation, and inappropriately cheerful. This clinical course is similar to observations in schizophrenic patients following prefrontal leukotomy, although their lesions were more extensive and not systematically reviewed. Yet, VMPFC trauma in otherwise healthy people has been shown to result in increased aggression. We hypothesize this patient’s pathologic aggression reflected developmental VMPFC dysfunction, and his subsequent VMPFC trauma manifest as indifference and joviality, as seen in the more extensive leukotomy lesions. Alternatively, VMPFC damage may result in a broad spectrum of pathologic behavior, as a consequence of emotional deregulation. Further investigation is required.

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