

# 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.<sup>1</sup> The protein colocalizes with the A $\beta$  peptide in brain amyloid deposits in patients with Alzheimer disease (AD), in senile plaque, and in vessel walls.<sup>2</sup> At least four polymorphisms have been described: two in the 5'-untranslated region and in exons 1 and 2.<sup>1</sup>

The gene for cystatin C is situated on chromosome 20, 20p11.2. It is 7.3 kb long and consists of three exons.<sup>3</sup> 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.<sup>1,4-6</sup> The GG genotype doubles the risk in patients at ages 80 and older.<sup>6</sup> Subsequent studies did not replicate this finding or the associated risk with the A allele and at younger ages.<sup>7-9</sup>

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  $71 \pm 8$  years, 142 older than 65 years) and 141 spouse control subjects (white; 83 female, 58 male; average age at onset  $72 \pm 8$  years, 120 older than 65 years). The clinical diagnosis of probable AD was made according to National Institute of Neurological and Communicative Diseases

and Stroke/Alzheimer's Disease and Related Disorders Association criteria (see reference E-1 on the *Neurology* Web site at [www.neurology.org](http://www.neurology.org)). We also included a CT scan or MRI or both that showed cortical atrophy. Each participant or authorized representative gave written informed consent for the study in accordance with institutional review board guidelines. Enrollment occurred in Texas and Georgia; additional cases were obtained from the National Cell Repository. 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.<sup>4</sup> 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  $\chi^2$  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 LOGISTIC 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  $\epsilon 24$ ,  $\epsilon 34$ , or  $\epsilon 44$  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

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**Table 1** CST 3 genotypes

	Patients	Controls
AA	17 (9.5)	6 (4.3)
AG	56 (31.3)	37 (26.2)
GG	106 (59.2)	98 (69.5)
A	90 (25.1)*	49 (17.4)*
G	268 (74.9)*	233 (82.6)*
A-	73 (40.8)†	43 (30.5)†
GG	106 (59.2)†	98 (69.5)†

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.

referent value of 1 and the respective 95% CI included 1. As expected, *APOE4* alone was a risk factor: 8-fold for men and 10-fold for women. The combination of CST 3 A and *APOE4* posed a high risk: 14-fold for men and 16-fold for women. These risks apply to risk at ages 65 and older and to a shift in risk from ages 65 and older to younger ages.

**Discussion.** The literature on the association of the G/A polymorphism in CST 3 with AD is difficult to interpret. The initial report identified the GG genotype with AD risk for survivors to ages 80 years and older ( $p = 0.04$ ).<sup>6</sup> Subsequently, a two-loci haplotype labeled B for promoter region and exon 1 G/A polymorphisms ( $n = 907$ )<sup>1</sup> demonstrated a fourfold elevated risk, higher at age 75 years and older. A Japanese sample did not indicate a statistically significant association for CST 3 AA/AG or for *APOE4* (low frequency among Japanese).<sup>8</sup> German samples also yielded negative results for late-onset AD.<sup>7</sup> However, a Spanish sample associated the CST 3 A allele with a threefold elevated risk before age 70 and eightfold for *APOE4* carriers; the G allele increased the risk in patients with an age at onset older than 80.<sup>10</sup>

Our results in a sample including early- and late-onset cases demonstrate elevated risk for CST 3 A

**Table 2** CST 3 genotypes in *APOE4* carriers

	Patients	Controls
All <i>APOE4</i> carriers		
A-	56 (45.2)*	9 (23.7)*
GG	68 (54.8)*	29 (76.3)*
Female <i>APOE4</i> carriers		
A-	42 (49.4)†	5 (20.8)†
GG	43 (50.6)†	19 (79.2)†

Values in parentheses are percentages.

\*  $p = 0.0181$ ; OR = 2.7; 95% CI = 1.2–6.1; standard power = 0.9368.

† Fischer's exact  $p = 0.0185$ ; OR = 3.7; 95% CI = 1.3–10.9; standard power = 0.9623.

**Table 3** Relative risk of Alzheimer disease

Gender and genotype	Odds ratio	95% Wald CI
Male	0	n/a
Male/ <i>APOE4</i> +	8.0	3–23
Male/CST 3 A+	1.2	0.4–4
Male/CST 3 A+/ <i>APOE4</i> +	14	4–50
Female	0.7	0.5–1
Female/ <i>APOE4</i> +	10	4–26
Female/CST 3 A+	2	0.7–6
Female/CST 3 A+/ <i>APOE4</i> +	16	6–45

Proportional odds model used; comparison with Male/*APOE4*-/CST3A-.

for *APOE4* carriers, 14-fold for men and 16-fold for women, in a proportional odds model designed to demonstrate risk of late-onset AD with a shift in risk to ages younger than 65. The magnitude of 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 CST 3 A was informative. However, the possibility of another polymorphism in linkage disequilibrium cannot be eliminated. Nonetheless, the involvement of additional risk factors in the disease process is a subject of intense interest.

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## 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.<sup>1</sup> 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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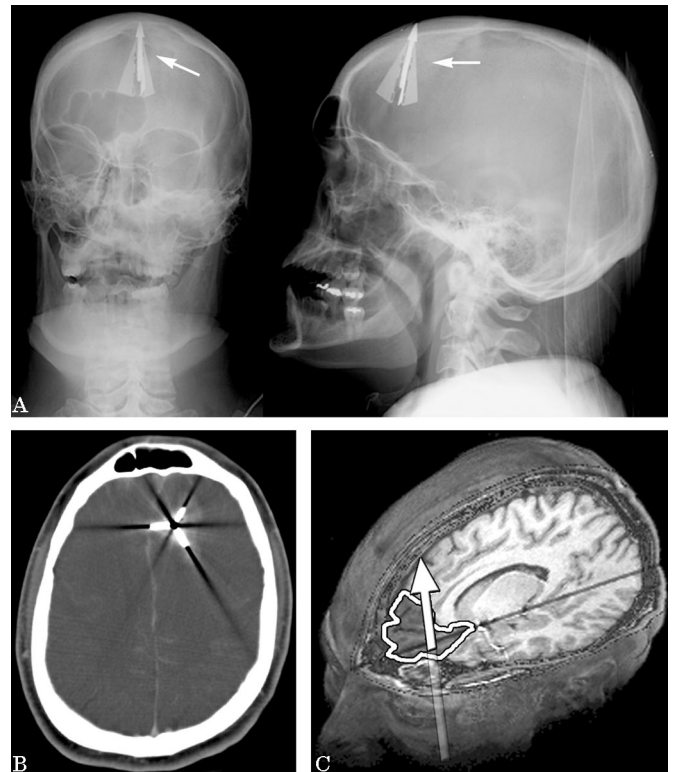


Figure. (A) Skull x-rays (anteroposterior and lateral) demonstrating crossbow bolt (arrows) lodged at the inner table of the frontal calvarium. (B) Enhanced CT scan illustrating metallic bolt in the left medial frontal lobe with surrounding hematoma and mild falx displacement. (C) MRI, normal brain. Superimposed arrow demonstrates the bolt's trajectory through the ventromedial prefrontal cortex (VMPFC). The normal VMPFC (shaded region) is comprised of Brodmann areas 10–13, 25, and 32, which include the medial orbital gyri, gyrus rectus (not shown), and inferior half of the medial prefrontal surface.<sup>2</sup>