Increased Risk for Alzheimer Disease With the Interaction of MPO and A2M Polymorphisms

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**Background:** The genes encoding myeloperoxidase (MPO) and α-2-macroglobulin (A2M) are involved in molecular pathways leading to β-amyloid deposition. Two polymorphic sites in these genes (MPO-G/A and A2M-Ile/Val) have been associated with Alzheimer disease (AD), but conflicting findings have been reported in populations with different ethnic backgrounds.

**Objectives:** To study the association of MPO-G/A and A2M-Ile/Val polymorphisms with sporadic AD and to investigate the interactions among the MPO, A2M, and apolipoprotein E (APOE) gene polymorphisms in determining the risk of the development of AD.

**Design:** Case-control study.

**Setting:** Referral center for AD in Calabria, southern Italy.

**Participants:** One hundred forty-eight patients with sporadic AD and 158 healthy control subjects.

**Results:** The MPO-G and A2M-Val alleles were found more frequently in cases than in controls, as were the MPO-G/G and A2M-Val/Val genotypes. The odds ratio (OR) for the MPO-G/G genotype was 1.78 (95% confidence interval [CI], 1.13-2.80); for the A2M-Val/Val genotype, 3.81 (95% CI, 1.66-8.75). The presence of MPO-G/G and A2M-Val/Val genotypes synergistically increased the risk of AD (OR, 25.5; 95% CI, 4.65-139.75). Stratification of cases by sex, age at onset of AD, and APOE-e4 status did not show significant differences in the distribution of MPO or A2M polymorphisms.

**Conclusions:** The MPO and A2M polymorphisms are associated with sporadic AD in southern Italy. Moreover, a genomic interaction between these polymorphisms increases the risk of the development of AD.

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**METHODS**

**Patients and Control Subjects**

Subjects included in this study were 148 patients with sporadic AD (90 women and 58 men, mean age 75.3 ± 8.8 years) and 158 healthy control subjects (79 women and 79 men, mean age 68.4 ± 8.1 years). The AD patients were recruited from the Neurological Sciences Research Unit, Department of Neurological Sciences, National Research Council, Piano Lago–Cosenza, Italy (Drs Zappia, Gambardella, and Quattrone); and the Institute of Neurological Sciences, National Research Council, Piano Lago–Cosenza, Italy (Drs Zappia, Mannina, Serra, Cittadella, Andreoli, La Russa, Annesi, Spadafora, Romeo, Nicoletti, Messina, Gambardella, and Quattrone).

**GENETIC FACTORS ARE INCREASINGLY RECOGNIZED AS PATHOGENIC DETERMINANTS IN SPORADIC ALZHEIMER DISEASE (AD).** To date, the e4 allele of the apolipoprotein E gene (APOE) polymorphism is considered the most important genetic risk factor for sporadic AD. The e4 allele is associated with a dose-dependent increase in the incidence of AD, and its presence correlates with the amount of β-amyloid (Aβ) deposition in the plaques. Aside from APOE, other genes coding for proteins involved in the deposition and clearance of Aβ could be relevant to the pathogenesis of sporadic AD. Among these, the genes encoding myeloperoxidase (MPO) and α-2-macroglobulin (A2M) may be of interest. The MPO gene encodes for an enzyme that catalyzes a reaction between hydrogen peroxide and chloride to generate hypochlorous acid, a potent oxidant leading to oxidizing conditions that are known to increase Aβ deposition, whereas the A2M gene encodes for a serum pan–protease inhibitor involved in the clearance of Aβ. Moreover, an MPO promoter polymorphism and an A2M polymorphism located in exon 24 have been identified as risk factors for AD, even if conflicting results have been reported in different populations. On these grounds, it has been hypothesized that APOE, MPO, and A2M polymorphisms may be mechanistically linked in a pathway leading to Aβ deposition.

In view of this hypothesis and also considering the conflicting findings reported in different populations, we investigated the association of MPO and A2M polymorphisms with sporadic AD in southern Italy and the interaction among APOE, MPO, and A2M polymorphisms in determining the risk of development of AD.
men; mean ± SD age, 71.3 ± 7.4 years) and 158 control subjects (85 women and 73 men; mean ± SD age, 74.1 ± 6.7 years). All case and control subjects were white and born in Calabria, southern Italy. Informed consent was obtained from all participants or from their relatives.

Patients were diagnosed as having probable AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association, and they were consecutively selected from among the outpatients attending the Institute of Neurology of the University Magna Graecia of Catanzaro, Italy, from September 1, 1999, until June 30, 2002. We included only patients with sporadic AD, ie, patients without any ascertained family history of AD, including in-first- and second-degree relatives, as determined by interviewing the patients and their relatives. Fifty patients had early-onset AD, ie, younger than 65 years (mean ± SD age at onset, 59.4 ± 5.3 years), and 98 patients had late-onset AD (mean ± SD age at onset, 73.0 ± 4.4 years). The age at onset of AD symptoms was estimated from semistructured interviews with the patients’ caregivers. For the control group, we used healthy subjects enrolled during a previous study on aging who underwent evaluation by a neurologist. We assessed their cognitive conditions with the Mini-Mental State Examination (MMSE). The MMSE scores were adjusted for age and education12; no healthy subject had an MMSE score below the 25th percentile for its adjusted norms (mean ± SD MMSE score, 25.0 ± 3.4).

MOLECULAR GENETIC ANALYSIS

The following 3 polymorphisms were examined at the MPO, A2M, and APOE loci: the biallelic (G/A) polymorphism located in the promoter region of the MPO gene (MPO-G/A polymorphism); the polymorphism located in exon 24 of the A2M gene with a missense mutation leading to a valine-to-isoleucine change at codon 1000 (A2M-Ile/Val polymorphism); and the APOE-e2/e3/e4 polymorphism. The AD and control DNA samples were provided as a masked, randomly selected set, 73.0±4.4 years). The age at onset of AD symptoms was estimated through calculation of odds ratios (ORs) with 95% confidence intervals (CIs). Furthermore, multiple logistic regression analysis was used to calculate ORs by adjusting the model for age, sex, and APOE-e4 status. Logistic regression analysis was used to test the interaction among MPO, A2M, and APOE polymorphisms.

RESULTS

Allele and genotype distributions for MPO-G/A and A2M-Ile/Val polymorphisms are given in Table 1. The observed genotype frequencies did not differ from the expected frequencies according to the Hardy-Weinberg equilibrium for the control (MPO-G/A, χ²=0.27 [P=.61]; A2M-Ile/Val, χ²=0.99 [P=.32]) and AD (MPO-G/A, χ²=3.13 [P=.08]; A2M-Ile/Val, χ²=3.53 [P=.06]) groups. The finding of MPO-G and A2M-Val alleles occurred significantly more frequently in cases than in controls, as did that of the genotypes MPO-G/G and A2M-Val/Val. Stratification of cases by sex, age at onset of AD, and APOE-e4 status did not show any significant differences in the allele and genotype distributions for the MPO-G/A polymorphism or for the A2M-Ile/Val polymorphism (data not shown).

Table 2 shows the risk for AD associated with MPO and A2M polymorphisms. When the risk was considered for a single polymorphism, the subjects who carried the genotype MPO-G/G had a 1.78-fold increased risk for AD (χ²=6.30 [P=.01]), and the subjects with the genotype A2M-Val/Val had a risk of 3.81 (χ²=11.11 [P<.001]). Logistic regression analysis, adjusting the model for age, sex, and APOE-e4 status, showed that the adjusted ORs were similar to the crude estimates. However, when the risk was considered by analyzing the coexistence of both MPO-G/G and A2M-Val/Val genotypes, the risk for AD was 25.5 for the subjects harboring both genotypes.
Our findings demonstrate that MPO and A2M genes are susceptibility loci for AD in a southern Italian population. Variations in the MPO-G/A and A2M-Ile/Val polymorphisms were associated with AD, independent of the APOE-ε4 status, and individuals harboring both the MPO-G/G and A2M-Val/Val genotypes had a much higher risk of AD than those harboring only 1 of these genotypes. This synergistic effect was not simply additive, and the cumulative risk of AD derived from carrying both genotypes was due to an interaction between polymorphisms. Our data suggest that this genomic interaction may be one of the underlying mechanisms of AD etiology, possibly by facilitating Aβ deposition in the brain of patients with AD or by decreasing its clearance.

We have previously reported the association with AD of 2 polymorphisms located in the A2M gene and in linkage disequilibrium between them, ie, the A2M-Ile/Val polymorphism and a 5-bp insertion/deletion polymorphism in intron 17 at the splice acceptor site of exon 18 (A2M-ins/del).12 In the present study, besides the MPO and APOE polymorphisms, we investigated only 1 of the 2 A2M polymorphisms, the A2M-Ile/Val polymorphism, because the main purpose of the present study was to investigate the interaction of several polymorphisms for the risk of AD, and thus it seemed more reasonable to study the A2M polymorphism theoretically more implicated in AD pathogenesis. Indeed, a significant increase of Aβ deposition has been shown in the brains of patients with AD bearing the A2M-Val allele.15

### Table 1. MPO-G/A and A2M-Ile/Val Allele and Genotype Distributions in Patients With AD and Control Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>No. (%) of MPO Alleles</th>
<th>No. (%) of MPO Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G          A          P Value</td>
<td>G/G        G/A        A/A      P Value</td>
</tr>
<tr>
<td>AD (n = 148)</td>
<td>226 (77.0) 68 (23.0) .002</td>
<td>84 (56.8) 60 (40.5)  4 (2.7) .004</td>
</tr>
<tr>
<td>Controls (n = 158)</td>
<td>208 (65.8) 108 (34.2)</td>
<td>67 (42.4) 74 (46.8)  17 (10.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>No. (%) of A2M Alleles</th>
<th>No. (%) of A2M Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ile        Val        P Value</td>
<td>Ile/Ile     Ile/Val     Val/Val      P Value</td>
</tr>
<tr>
<td>AD (n = 148)</td>
<td>188 (63.5) 108 (36.5) .004</td>
<td>65 (43.9) 58 (39.2)  25 (16.9) .003</td>
</tr>
<tr>
<td>Controls (n = 158)</td>
<td>236 (74.4) 81 (25.6)</td>
<td>85 (53.8) 65 (41.1)  8 (5.1)</td>
</tr>
</tbody>
</table>

*Indicates a crude estimate.
†Indicates estimate adjusted for apolipoprotein ε4 status, age, and sex using logistic regression.

**Abbreviations:** AD, Alzheimer disease; A2M, the gene encoding α2-macroglobulin; MPO, the gene encoding myeloperoxidase.

### Table 2. Estimated OR for AD Development Associated With Isolated MPO or A2M Polymorphisms and With the Coexistence of Both Polymorphisms in 148 Patients With AD and 158 Control Subjects

<table>
<thead>
<tr>
<th>Polymorphisms, Genotype</th>
<th>No. (%) of Subjects</th>
<th>AD Group</th>
<th>Control Group</th>
<th>OR† (95% CI) Adjusted OR‡ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>84 (56.8)</td>
<td>67 (42.4)</td>
<td>1.78 (1.13-2.80)</td>
<td>1.65 (1.01-2.71)</td>
</tr>
<tr>
<td>A+</td>
<td>64 (43.2)</td>
<td>91 (57.6)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>A2M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>25 (16.9)</td>
<td>8 (5.1)</td>
<td>3.81 (1.66-8.75)</td>
<td>3.58 (1.46-8.77)</td>
</tr>
<tr>
<td>Ile+</td>
<td>123 (83.1)</td>
<td>150 (94.9)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>MPO and A2M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G and Val/Val</td>
<td>17 (11.5)</td>
<td>1 (0.6)</td>
<td>25.5 (4.65-129.75)</td>
<td>23.19 (2.86-188.3)</td>
</tr>
<tr>
<td>G/G and Ile+</td>
<td>67 (45.3)</td>
<td>66 (41.8)</td>
<td>1.52 (0.94-2.46)</td>
<td>1.40 (0.83-2.36)</td>
</tr>
<tr>
<td>A+ and Val/Val</td>
<td>8 (5.4)</td>
<td>7 (4.4)</td>
<td>1.71 (0.61-4.84)</td>
<td>1.52 (0.47-4.93)</td>
</tr>
<tr>
<td>A+ and Ile+</td>
<td>56 (37.8)</td>
<td>84 (53.2)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
</tbody>
</table>

*Indicates estimate adjusted for apolipoprotein ε4 status, age, and sex using logistic regression.
†Indicates a crude estimate.
‡Indicates estimate adjusted for apolipoprotein ε4 status, age, and sex using logistic regression.

*χ² = 19.59 [P < .001]. This effect was due to an interaction between the MPO-G/G and A2M-Val/Val genotypes (interaction term by using logistic regression analysis, G = 4.59 [P = .03]). When analyzing the distribution of the APOE polymorphism by considering the subjects carrying at least 1 APOE-ε4 allele (cases, 64/148 [43.2%]; controls, 17/158 [10.8%]; χ² = 41.43 [P < .001]; OR, 6.32 [95% CI, 3.47-11.51]), no significant interactions were observed between the APOE-ε4 carrier status and the MPO-G/G (G = 2.097 [P = .15]) or A2M-Val/Val genotype (G = 1.243 [P = .27]).
Biological evidence also supports a role in the pathogenesis of AD for the MPO-G/A polymorphism. This promoter polymorphism regulates MPO gene expression, because the G allele, at variance with the A allele, creates an SP1 transcription site associated with stronger promoter activity and thus provided a larger amount of myeloperoxidase than the A allele. The deposition of Aβ increases in oxidizing conditions, and the presence of myeloperoxidase could enhance these conditions through production of hypochlorous acid, a potent oxidant that reacts with superoxide to generate highly reactive hydroxyl radicals. Myeloperoxidase is absent in normal brain parenchyma, but it is found with immunodetection methods in activated microglial macrophages surrounding amyloid plaques and in the plaques of patients with AD, colocalizing with Aβ1-42. Moreover, treatment of rodent microglia with aggregated Aβ1-42 was shown to induce MPO messenger RNA expression, which suggests that the MPO gene is apparently maintained in a dormant state in quiescent microglia and reactivated in response to certain signals, such as Aβ deposition.

These observations indicate that myeloperoxidase may participate in AD pathologic manifestations through oxidation of Aβ and suggest that an enhanced MPO gene expression, such as that regulated by the G allele of the MPO-G/A polymorphism, may increase Aβ deposition. Nevertheless, the findings of association studies have been variable. Indeed, one study showed an overrepresentation of the MPO-G/G genotype in women with AD, but not in men; one study reported an increased risk of AD in white patients of both sexes carrying the MPO-G/G genotype, but not in Hispanics; another study in a Finnish population found that the MPO-A allele in association with the APOE-ε4 allele increased the risk of AD only in men; and, finally, a recent study in a large Spanish population failed to detect any association between the MPO polymorphism and AD. Certainly, differences in ethnic background and the composition of populations may explain the discrepancies among studies. Our present data add further evidence of the relevance of the putative role of the MPO polymorphism in AD pathogenesis, at least in a southern Italian population.

The most important finding of the present study, however, is the interaction we have demonstrated between MPO-G/A and A2M-Ile/Val polymorphisms in determining the risk of AD. To our knowledge, this genomic interaction produced the highest genetic risk factor for sporadic AD reported in the literature. We are aware that our data should be interpreted with caution, because our stratified analyses on the combination of 2 polymorphisms included small sample sizes. Nevertheless, we believe that our finding of the genomic interaction for AD in individuals bearing the MPO-G/G and A2M-Val/Val genotypes is important and should be confirmed by separate investigations in different populations.

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Author contributions: Study concept and design (Drs Zappia and Quattrone); acquisition of data (Drs Andreoli, Spadafora, Romeo, Nicoletti, Messina, and Gambardella); analysis and interpretation of data (Drs Zappia, Manna, Serra, Citadella, Andreoli, La Russa, Annesi, Spadafora, and Quattrone); drafting of the manuscript (Drs Zappia, Manna, and Quattrone); critical revision of the manuscript for important intellectual content (Drs Zappia, Manna, Serra, Citadella, Andreoli, La Russa, Annesi, Spadafora, Romeo, Nicoletti, Messina, Gambardella, and Quattrone); statistical expertise (Drs Zappia, Serra, and Quattrone); administrative, technical or material support (Drs Manna, Nicoletti, and Messina); study supervision (Drs Zappia and Quattrone).

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