



ORIGINAL ARTICLE

Role of genes *GSTM1*, *GSTT1*, and *MnSOD* in the development of late-onset Alzheimer disease and their relationship with *APOE**4[☆]



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Received 3 May 2014; accepted 10 October 2014

Available online 30 August 2016

KEYWORDS

Polymorphism;
Alzheimer disease;
GSTM1;
GSTT1;
MnSOD;
Oxidative damage

Abstract

Introduction: Several studies have reported increased oxidation of lipids, proteins and DNA in the brains of patients with Alzheimer disease (AD). Moreover, these patients display differences in the activity and polymorphisms of the genes encoding the enzymes GST (T1, M1) and MnSOD. For these reasons, we designed a study of the variability in *GSTT1*, *GSTM1*, and *MnSOD* genes in healthy and AD groups from a Venezuelan population.

Methods: We included 179 unrelated Venezuelan subjects classified as either AD patients ($n=79$) or healthy individuals ($n=100$). Presence or absence of the *GSTT1/GSTM1* genes was determined using PCR-SSP, and polymorphisms of *MnSOD* and *APOE* genes were identified with PCR-RFLP.

Results: The genotype *GSTT1+/GSTM1-* seems to favour development of AD (OR=2.06, $P=.01$). The risk level is higher when it is combined with the $\epsilon 4$ allele of the *APOE* gene: *GSTT1+/GSTM1-/\epsilon 3\epsilon 4* (OR=3.07, $P=.05$), *GSTT1+/GSTM1-/\epsilon 4\epsilon 4* (OR=5.52, $P=.02$). The *Ala-9Val* polymorphism does not appear to be related to AD. However, the presence of the *Ala/Ala* genotype increases the risk provided by the $\epsilon 4$ allele of the *APOE* gene: *AlaAla/\epsilon 3\epsilon 4* (OR=3.47, $P=.03$), *AlaAla/\epsilon 4\epsilon 4* (OR=6.3, $P=.01$).

Conclusions: The results support the hypothesis that impaired mitochondrial function and increased oxidative damage are involved in the pathogenesis of AD. It is important to study other genes related to oxidative stress and antioxidant pathways which could be involved in susceptibility to AD.

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[☆] Please cite this article as: de Mendonça E, Salazar Alcalá E, Fernández-Mestre M. Papel de las variantes *GSTM1*, *GSTT1* y *MnSOD* en el desarrollo de enfermedad de Alzheimer de aparición tardía y su relación con el alelo 4 de *APOE*. Neurología. 2016;31:535–542.

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PALABRAS CLAVE

Polimorfismo;
Alzheimer;
GSTM1;
GSTT1;
MnSOD;
Daño oxidativo

Papel de las variantes *GSTM1*, *GSTT1* y *MnSOD* en el desarrollo de enfermedad de Alzheimer de aparición tardía y su relación con el alelo 4 de *APOE*

Resumen

Introducción: Diversos estudios han descrito que en los cerebros de pacientes con enfermedad de Alzheimer (EA) hay una mayor oxidación de lípidos, proteínas y ADN. Además, en estos pacientes se ha observado diferencias en la actividad y polimorfismos de los genes que codifican las enzimas GST (T1 y M1) y MnSOD. En virtud de ello se planteó estudiar la variabilidad de los genes *GSTT1*, *GSTM1* y *MnSOD* en individuos venezolanos sanos y con EA.

Métodos: Se incluyeron 179 individuos venezolanos, no relacionados, agrupados en pacientes con EA (n = 79) e individuos sanos (n = 100). La presencia o ausencia de los genes *GSTT1/GSTM1* se determinó por PCR-SSP y los polimorfismo de los genes *MnSOD* y *APOE* por PCR-RFLP.

Resultados: El genotipo *GSTT1+/GSTM1-* parece favorecer el desarrollo de la EA (OR = 2,06; p = 0,01), siendo el riesgo mayor al estar en combinación con el alelo $\epsilon 4$ del gen *APOE*: *GSTT1+/GSTM1-/\epsilon 3\epsilon 4* (OR = 3,07; p = 0,05), *GSTT1+/GSTM1-/\epsilon 4\epsilon 4* (OR = 5,52; p = 0,02). El polimorfismo *Ala-9Val* por sí solo no parece estar relacionado con la EA, sin embargo, la presencia del genotipo *Ala/Ala* incrementa el riesgo que proporciona el alelo $\epsilon 4$ del gen *APOE*: *AlaAla/\epsilon 3\epsilon 4* (OR = 3,47; p = 0,03), *AlaAla/\epsilon 4\epsilon 4* (OR = 6,3; p = 0,01).

Conclusiones: Los resultados apoyan la hipótesis de que el deterioro de la función mitocondrial y el aumento de daño oxidativo están involucrados en la patogénesis de la EA. Es importante estudiar otros genes relacionados con estrés oxidativo y vías antioxidantes, los cuales pudiesen estar involucrados en la susceptibilidad a desarrollar la EA.

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Introduction

Neurodegenerative diseases, including Alzheimer disease (AD), display oxidative damage as a common feature, but it is unclear whether this process is the cause or the effect of the disease.¹ Oxidative damage may be caused by the peptide amyloid β (A β) and the paired helical filaments of tau protein that constitute the typical lesions in AD. The A β peptide has been observed to give rise to free radicals² and inhibit the cytochrome oxidase enzyme. This contributes to oxidative stress.³ Observations have also shown that the paired helical filaments of tau protein may form advanced glycation end-products, and this generates enough reactive oxygen species (ROS) to cause oxidative damage.⁴ Since the brain is a major consumer of oxygen with a high demand for energy and only limited antioxidant ability compared to other tissues, it is very susceptible to oxidative damage. Antioxidant defences therefore play a vital role as the means of eliminating free radicals.⁵ Patients with AD have displayed altered activity of glutathione S-transferase enzymes (GSTs) and manganese superoxide dismutase (MnSOD).⁶ GSTs are a family of enzymes that are crucial to the protection of cells against toxic substances and oxidative stress. They are encoded by some 16 genes, further subdivided into 8 classes.⁷ The μ class comprises 5 different isoenzymes named *GSTM1* through *GSTM5*. The θ class includes only 2 isoenzymes, *GSTT1* and *GSTT2*. The null genotypes of genes *GSTM1* and *GSTT1* are characterised by homozygous knockout of the gene, resulting in absent enzyme activity (overview by Cooper⁸). Both the *GSTT1* and *GSTM1* enzymes are known for their ability to catalyse the detoxification of reactive oxygen and products of lipid peroxidation.⁹ Because

of this, inactivity of the *GSTT1/M1* enzymes has been linked to greater exposure to oxidative stress.¹⁰

The superoxide dismutase enzyme (SOD) is one of the main defences against the damage that can be caused by the free radical superoxide (O $_2^{\cdot-}$). The enzyme catalyses the conversion of superoxide into molecular oxygen (O $_2$) and hydrogen peroxide (H $_2$ O $_2$); these molecules will later be converted into water by the action of catalase or glutathione peroxidase enzymes.¹¹ Superoxide dismutase exists in 3 isoforms: cytosolic [Cu/ZnSOD], extracellular [EC-SOD], and mitochondrial [MnSOD].¹² Since 90% of all ROS originate in the mitochondria, MnSOD is an antioxidant which plays a fundamental role in protecting cells against oxidative stress. The activity of this mitochondrial enzyme defends cells from lipid peroxidation; within the brain, it protects the viability of the neuronal membrane.¹³ MnSOD enzyme is synthesised in the cytosol and then transported to the mitochondria. MnSOD transport requires the participation of a sequence of 24 amino acids known as the mitochondrial targeting sequence (MTS). These amino acids form the amphiphilic helices required to transport the enzyme to the mitochondria.¹⁴ The gene that encodes MnSOD possesses a polymorphism by which thymine (T) is replaced by cytosine (C) at nucleotide 47, resulting in an amino acid substitution (Val \rightarrow Ala) at position -9 of the MTS and altering the enzyme structure. These changes in the structure alter mitochondrial transport of MnSOD, and therefore affect the cell's ability to defend itself from superoxide.¹⁴ Multiple studies have now demonstrated the role MnSOD plays in how neurons survive oxidative stress.^{15,16} Considering that *GSTT1*, *GSTM1*, and *MnSOD* enzymes contribute to cell defence against oxidative stress, and the possibility that oxidative

stress and AD pathogenesis are related, we aim to study the role of polymorphisms of the genes *GSTT1*, *GSTM1* and *MnSOD* in the development of AD and correlate any such associations with the presence of allele $\epsilon 4$ of the *APOE* gene.

Materials and methods

Patients

The study was carried out in 79 patients (mean age, 70 ± 10 years), all of whom had been diagnosed with sporadic AD. All patients had received treatment in the neurology department at Hospital Clínico Universitario de Caracas (Venezuela) at some time between September 2004 and October 2006. These patients were selected according to the clinical protocol implemented in the Luis Borges Neuropsychology Unit of the neurology department at Hospital Clínico Universitario de Caracas. The protocol was framed in accordance with the specifications of the American Psychiatric Association (DSM-IV) and the NINCDS-ADRDA Alzheimer's Criteria (National Institute of Neurological Disorders, Communicative Disorders, and Stroke; Alzheimer's Disease and Related Disorders Association).

The control group consisted of 100 healthy Venezuelan residents with a mean age of 71 ± 10 years. These subjects completed the Mini-Mental State Examination and laboratory and imaging studies.

All participants in the study gave their informed consent; the consent of participants with AD was authorised by their legal guardians. The consent procedure was approved by the bioethics committees at Instituto Venezolano de Investigaciones Científicas and Hospital Clínico Universitario de Caracas.

Genomic DNA extraction

Genomic DNA was extracted from leukocytes and lymphocytes in peripheral blood according to Bunce's method.¹⁷

Detection of *GSTT1/GSTM1* genes

A multiplex PCR protocol was used to determine presence or absence of the *GSTM1* and *GSTT1* genes according to a modified version of the method described by Lin et al.¹⁸ and the specific initiators used by Chen et al.¹⁹

Genotyping of the *MnSOD* gene

The *Ala-9Val* polymorphism was studied using the PCR-RFLP method with the initiators and protocol described by Ambrosone et al.²⁰

Genotyping of the *APOE* gene

Researchers genotyped and evaluated the *APOE* polymorphism using PCR-RFLP with the initiators described by

Emi et al.²¹ and the protocol published by Hixson and Vernier.²²

Statistical analysis

Genotypic and allelic frequencies were calculated. The statistical significance of intergroup differences in frequency (for alleles, genotypes, and genotypic combinations) was estimated using the Mantel–Haenszel chi-square statistic and 2×2 contingency tables. Researchers adjusted *P*-values by multiplying them by the number of completed comparisons (Bonferroni correction). Differences were considered statistically significant for $P < .05$.

Results

Genotype distribution and genotypic combination of *GSTT1* and *GSTM1* genes in healthy individuals and patients with Alzheimer disease

Table 1 displays the distribution of the *GSTM1* (wild-type [+], null [–]) and *GSTT1* (wild-type [+], null [–]) in healthy individuals and patients with AD. The comparison of frequencies revealed the wild-type *GSTM1* genotype to be significantly more frequent in controls than in patients (OR: 0.58; 95% CI: 0.3235–1.0659; $P = .04$; $P_{corr} = ns$). In contrast, the null *GSTM1* genotype had a significantly higher frequency in patients than in controls (OR: 1.7; 95% CI: 0.9381–3.0903; $P = .04$; $P_{corr} = ns$). Nevertheless, differences were not statistically significant once *P*-values were corrected.

The study of genotype combinations for *GSTT1* and *GSTM1* found that all possible combinations were present in both groups. The *GSTT1+ / GSTM1–* combination was the most frequent in patients with AD, followed by the combinations *GSTT1+ / GSTM1+*, *GSTT1– / GSTM1+*, and *GSTT1– / GSTM1–*. The *GSTT1+ / GSTM1+* combination was the most frequent among healthy controls, followed by the combinations *GSTT1+ / GSTM1–*, *GSTT1– / GSTM1+*, and *GSTT1– / GSTM1–*. The comparison of frequencies revealed the combination *GSTT1+ / GSTM1–* to be significantly more frequent in patients than in controls (OR: 2.06; 95% CI: 1.1118–3.8036; $P = .09$; $P_{corr} = .036$). The *P*-value remained significant after correction (Table 1).

Allelic and genotypic frequencies of the *MnSOD* gene in healthy individuals and patients with Alzheimer disease

In both groups, the *Ala/Val* genotype had the highest frequency, followed by *Ala/Ala* and *Val/Val* in AD patients and *Val/Val* and *Ala/Ala* in healthy controls. The comparison of frequencies revealed the *Ala/Val* genotype to be significantly more frequent in patients than in controls (OR: 1.9; 95% CI: 0.9147–3.9498; $P = .04$; $P_{corr} = ns$). However, differences were not statistically significant once *P*-values were corrected (Table 2).

Table 1 Distribution of genotypes and genotype combinations for the genes *GSTT1* and *GSTM1* in patients with AD and healthy controls.

	AD <i>n</i> = 79	Healthy <i>n</i> = 100	OR 95% CI	<i>P</i>
<i>GSTT1</i> alleles				
Wild-type (+)	83.5 (66)	78 (78)	1.43	ns
Null (–)	16.5 (13)	22 (22)	0.69	ns
<i>GSTM1</i> alleles				
Wild-type (+)	46.8 (37)	60 (60)	0.58	.04
Null (–)	53.2 (42)	40 (40)	1.7	.04
<i>Allele combinations</i>				
<i>GSTT1</i> + <i>GSTM1</i> –	35.4 (28)	47 (47)	0.61	.08
<i>GSTT1</i> – <i>GSTM1</i> –	5.1 (4)	9 (9)	0.53	ns
<i>GSTT1</i> + <i>GSTM1</i> –	48.1 (38)	31 (31)	2.06	.009*
<i>GSTT1</i> – <i>GSTM1</i> +	11.4 (9)	13 (13)	0.86	ns

AD: Alzheimer disease; 95% CI: confidence interval; ns: not significant; OR: odds ratio.

Values shown in parentheses indicate the number of individuals bearing the genotype for the polymorphic site under study. Frequencies are expressed as percentages.

* Significant after applying the Bonferroni correction.

Allelic and genotypic frequency distributions of the *MnSOD* gene in healthy individuals and patients with Alzheimer disease

Table 3 displays the genotypes of the *APOE* gene for AD patients and healthy controls. The frequencies of genotypes $\epsilon_2\epsilon_3$ (OR = 0.04; 95% CI: 0.002-0.764; *P* = .0007; *P*corr = .0035) and $\epsilon_3\epsilon_3$ (OR = 0.55; IC 95%: 0.3073-1.0136; *P* = .02; *P*corr = ns) were significantly higher in controls than in AD patients. In contrast, patients displayed higher frequencies of genotypes $\epsilon_3\epsilon_4$ (OR = 1.93; 95% CI: 1.0164-3.6980; *P* = .02; *P*corr = ns) and $\epsilon_4\epsilon_4$ (OR = 4.29; 95% CI: 1.3241-13.9016; *P* = .004; *P*corr = .02). The comparison of allele frequencies also showed that ϵ_2 (OR = 0.04; 95% CI: 0.0059-0.3651; *P* = .000025; *P*corr = .000075) and allele ϵ_3 (OR = 0.5; 95% CI: 0.3220-0.8195; *P* = .0024; *P*corr = .0072)

were significantly more common among controls than in AD patients. Nevertheless, allele ϵ_4 was significantly more frequent in AD patients than in controls (OR = 2.8; 95% CI: 1.7003-4.6221; *P* = .000019; *P*corr = .000057).

Study of the combined effect of polymorphism in *GSTT1*, *GSTM1* and *APOE* genes

There are 24 possible combinations of *GSTT1*/*GSTM1*/*APOE*, and we observed 11 in the AD group and 15 in the controls. The frequencies of the combinations *GSTT1*+/*GSTM1*+/ $\epsilon_2\epsilon_3$ (OR = 0.1; 95% CI: 0.0059-2.004; *P* = .02; *P*corr = ns) and *GSTT1*+/*GSTM1*+/ $\epsilon_3\epsilon_3$ (OR = 0.46; 95% CI: 0.2114-1.002; *P* = .02; *P*corr = ns) were significantly higher in controls than

Table 2 Allelic and genotypic frequency of the *Ala-9Val* polymorphism of the *MnSOD* gene in patients and controls.

	AD <i>n</i> = 79	Healthy <i>n</i> = 100	OR 95% CI	<i>P</i>
<i>Genotypes</i>				
<i>Ala/Ala</i>	26.6 (21)	16 (16)	1.9	.04
<i>Ala/Val</i>	54.4 (43)	63 (63)	0.7	ns
<i>Val/Val</i>	19 (15)	21 (21)	0.88	ns
<i>Alleles</i>				
<i>Ala</i>	53.8 (85)	47.5 (95)	1.28	ns
<i>Val</i>	46.2 (73)	52.5 (105)	0.77	ns

AD: Alzheimer disease; 95% CI: confidence interval; ns: not significant; OR: odds ratio.

Values shown in parentheses indicate the number of repeats of the allele or the number of individuals bearing the genotype for the polymorphic site under study. Frequencies are expressed as percentages.

Table 3 Frequencies of genotypes and alleles of the *APOE* gene in patients with AD and controls.

	AD <i>n</i> = 79	Controls <i>n</i> = 100	OR 95% CI	<i>P</i>
<i>Genotype</i>				
ϵ_2/ϵ_3	0 (0)	12 (12)	0.04	.0007
ϵ_2/ϵ_4	1.3 (1)	0 (0)	3.84	ns
ϵ_3/ϵ_3	45.6 (36)	60 (60)	.55	.02
ϵ_3/ϵ_4	37.9 (30)	24 (24)	1.93	.02
ϵ_4/ϵ_4	15.2 (12)	4 (4)	4.29	.004
<i>Alleles</i>				
ϵ_2	0.6 (1)	6 (12)	0.04	.00002
ϵ_3	64.6 (102)	78 (156)	0.513	.0024
ϵ_4	34.8 (55)	16 (32)	2.8	.000019

AD: Alzheimer disease; 95% CI: confidence interval; ns: not significant; OR: odds ratio.

Values in parentheses indicate the number of individuals bearing the genotype. Frequencies are expressed as percentages.

Table 4 Frequencies of genotype combinations for *GSTT1/GSTM1/APOE* in patients with AD and controls.

Genotype	AD n = 79	Controls n = 100	OR 95% CI	P (χ^2)
<i>GSTT1+/GSTM1+/ε2ε3</i>	0 (0)	5 (5)	0.10	.02
<i>GSTT1+/GSTM1+/ε3ε3</i>	14 (11)	26 (26)	0.46	.02
<i>GSTT1+/GSTM1-/ε3ε4</i>	14 (11)	5 (5)	3.07	.019
<i>GSTT1+/GSTM1-/ε4ε4</i>	10 (8)	2 (2)	5.52	.009

AD: Alzheimer disease; 95% CI: confidence interval; OR: odds ratio; χ^2 : chi-square test. Values in parentheses indicate the number of individuals bearing the genotype. Frequencies are expressed as percentages.

in patients. In contrast, frequencies of the combinations *GSTT1+/GSTM1-/ε3ε4* (OR = 3.07; 95% CI: 1.0210-9.2514; *P* = .019; *P*_{corr} = ns) and *GSTT1+/GSTM1-/ε4ε4* (OR = 5.52; 95% CI: 1.1381-26.7837; *P* = .009; *P*_{corr} = ns) were significantly higher in AD patients than in controls (Table 4).

Study of the combined effect of the polymorphism *Ala-9Val* in the genes *MnSOD* and *APOE*

Of the 18 possible combinations of *MnSOD/APOE*, 12 were observed in controls and 9 in patients. The combinations *AlaAla/ε3ε4* (OR = 3.4; 95% CI: 1.0476-11.5486; *P* = .016; *P*_{corr} = ns) and *AlaVal/ε4ε4* (OR = 7.9; 95% CI: 1.7027-36.8988; *P* = .001; *P*_{corr} = ns) were significantly more common in AD patients than in controls. We must point out that some genotype combinations were present only in the control group: *AlaVal/ε2ε3* (OR = 0.09; 95% CI: 0.0050-1.6480; *P* = .014; *P*_{corr} = ns), *AlaVal/ε3ε3* (OR = 0.5; 95% CI: 0.2642-0.9461; *P* = .016; *P*_{corr} = ns), and *ValVal/ε2ε3* (OR = 0.1; 95% CI: 0.0059-2.0049; *P* = .02; *P*_{corr} = ns) (Table 5).

Discussion

The imbalance between the production and the elimination of ROS and nitrogen is known as oxidative stress. The cell must overcome oxidative stress to restore redox balance and thereby avoid the neuronal death and loss of

neuronal function that are associated with neurodegenerative diseases.²³ Studies in the area of AD pathogenesis have focused on the role of oxidative stress since the central nervous system is a great consumer of energy with only minimal antioxidant defences, making it highly sensitive to oxidative stress.²⁴ In the brain with AD, antioxidant systems function less effectively, which may lead to an increase in the reactive species of oxygen and nitrogen that would react to biomolecules, including proteins, lipids, carbohydrates, DNA, and RNA. This would cause changes in their chemical structure, and in turn, loss of function.²⁵ Oxidative stress in the brains of AD patients is well-documented: researchers have observed high levels of antioxidant enzymes, as well as increased concentrations of oxidative stress markers in the brains of AD patients compared to those of age-matched controls. Furthermore, the role played by oxidative stress in AD progression was observed when we compared the presence of the same brain proteins with oxidative damage in subjects with mild cognitive impairment, early-stage AD, and late-stage AD. This demonstrates that certain major pathways are activated and may be involved in AD progression.²⁵ Given that the GST family of enzymes conjugates reduced glutathione with electrophilic compounds, thus helping remove it from cells to prevent oxidative damage,²⁶ several studies have been published on the genes that encode the *GSTM1* and *GSTT1* enzymes and their role in different diseases. These enzymes proceed from a null phenotype characterised by absence of enzymatic activity due to inherited homozygous elimination of the full gene. In this study, the null *GSTM1* genotype was significantly more

Table 5 Frequencies of genotype combinations of the *MnSOD/APOE* gene in patients with AD and controls.

Genotype	AD n = 79	Controls n = 100	OR 95% CI	P (χ^2)
<i>AlaAla/ε3ε4</i>	12.6 (10)	4 (4)	3.4	.03
<i>AlaVal/ε2ε3</i>	0 (0)	6 (6)	0.09	.03
<i>AlaVal/ε3ε3</i>	26.6 (21)	42 (42)	0.5	.02
<i>AlaVal/ε4ε4</i>	11.4 (9)	2 (2)	7.9	.01
<i>ValVal/ε2ε3</i>	0 (0)	5 (5)	0.1	.05

AD: Alzheimer disease; 95% CI: confidence interval; OR: odds ratio; χ^2 : chi-square test. Values in parentheses show the number of individuals bearing the genotype for the polymorphic site under study. Frequencies are expressed as percentages.

frequent in patients with AD (OR: 1.7; $P = .04$; $P_{\text{corr}} = \text{ns}$); the wild-type *GSTM1* genotype was significantly more common in healthy controls (OR=0.58; $P = .04$; $P_{\text{corr}} = \text{ns}$), in contrast with findings from other studies.^{9,27–30} Likewise, the combination *GSTT1+/GSTM1–* was more frequently seen in AD patients than in controls (OR: 2.06; $P_{\text{corr}} = .036$). These results suggest that the null genotype *GSTM1* and the combination *GSTT1+/GSTM1–* could confer up to twice the risk of developing AD. Considering that a variety of studies suggest that variations in the *APOE* gene may be genetic risk factors for AD,^{31–33} we recorded the combinations of *GSTT1/GSTM1* and *APOE* genotypes and obtained interesting results. The genotype combinations *GSTT1+/GSTM1–/ε3ε4* and *GSTT1+/GSTM1–/ε4ε4* were significantly more frequent in patients than in controls. These combinations may therefore be linked to increased likelihood of developing AD. Additionally, the risk posed by the presence of one or 2 $\epsilon 4$ alleles of the *APOE* gene is higher in the combination in which the *GSTM1* gene is absent. Considering the above, an increase in A β peptide aggregation due to the E4 isoform of apolipoprotein E,³⁴ together with the reduction in antioxidant defences caused by homozygotic elimination of the *GSTM1* gene, could generate greater oxidative stress. This in turn would give rise to more neuronal death and explain how the risk inherent to allele $\epsilon 4$ of the *APOE* gene would multiply in the absence of the *GSTM1* gene. Furthermore, combinations including wild-type *GSTT1* and *GSTM1* and at least one $\epsilon 3$ allele [*GSTT1+/GSTM1+/ε2ε3* and *GSTT1+/GSTM1+/ε3ε3*] were significantly more frequent in healthy controls than in AD patients. This suggests that these combinations may provide protection against the onset of AD. A β peptides are components of the senile plaques that begin degeneration of brain neurons in AD by increasing the presence of ROS until it exceeds the defence ability of each cell. Kaminsky and Kosenko³⁵ studied the effects of A β peptides on antioxidant enzymes from mitochondrial and non-mitochondrial sources in the rat brain in vivo. They observed that A β peptides increase the enzymatic activities creating H₂O₂ while inhibiting the activity of enzymes that consume H₂O₂ in the mitochondria and cytosol. This finding suggests that the imbalance between enzymes generating and those metabolising H₂O₂ contribute to the underlying oxidative stress in the neurodegeneration and neuronal death that occur in AD. Furthermore, the A β peptide has been found to be involved in the decreased expression of cytochrome c oxidase in the mitochondria, which in turn affects the electron transport chain and produces ROS.³ The MnSOD enzyme forms part of the cell's enzymatic antioxidant defences, and in fact provides the cell's first line of defence against the superoxide anion by converting it into H₂O₂.¹² Multiple studies now support that MnSOD plays a role in how neurons survive oxidative stress. One such study, performed in *MnSOD* gene-knockout mice, reported that the mice died not long after birth with signs of neurodegeneration.^{15,16} Similarly, studies in transgenic rats have reported that MnSOD deficiency increases the concentrations of the A β peptide, which favours the formation of senile plaques.³⁶ However, other studies have reported that overexpression of the MnSOD enzyme actually protects neurons from oxidative damage.^{37–39} In light of this conflicting evidence, we studied the *Ala-9Val* polymorphism of the *MnSOD* gene that alters the secondary

structure of that protein, and therefore the mitochondrial transport of MnSOD. This affects the cell's ability to defend itself from superoxide radicals.⁴⁰ A comparison of the frequencies of the *Ala-9Val* genotypes of *MnSOD* showed that genotype *Ala/Ala* was significantly more frequent in patients than in healthy controls. This finding does not coincide with the results from an Italian population reported by Ventriglia et al.¹³ Furthermore, evaluating the combined effect of the *MnSOD* and *APOE* genes revealed that the frequencies of the combinations *AlaAla/ε3ε4* (OR = 3.4; $P = .03$) and *AlaVal/ε4ε4* (OR = 7.9; $P = .01$) were significantly higher in patients than in controls, meaning that these combinations may indicate a greater susceptibility to AD. The presence of the *Ala* allele, which is associated with greater enzymatic activity of MnSOD in humans, as well as the presence of allele $\epsilon 4$, promotes the development of AD. We should point out that the risk associated with genotypes $\epsilon 3\epsilon 4$ and $\epsilon 4\epsilon 4$ (OR = 1.93 and OR = 4.29, respectively) is approximately twice as high when the genotypes *AlaAla* and *AlaVal* of the *MnSOD* gene are present (OR = 3.4 and OR = 7.9, respectively). In contrast, the genotypic combinations *AlaVal/ε2ε3*, *AlaVal/ε3ε3*, and *ValVal/ε2ε3* were only present in healthy individuals. This suggests that they may play a protective role against AD. Allele $\epsilon 3$, whether present homozygously or with allele $\epsilon 2$, together with one or 2 doses of the *Val* allele that is associated with abnormal enzymatic activity by MnSOD,^{40,41} would therefore protect individuals from developing AD. Considering that the *Ala* form of MnSOD is more efficiently transported to the mitochondria than the *Val* form,¹⁴ and that this enzyme catalyses the dismutation of the superoxide anion in H₂O₂ so that it can subsequently be removed from the mitochondria by the action of catalase and glutathione peroxidase, researchers have suggested that the increased enzymatic activity of MnSOD would result in more production of H₂O₂. This in turn would create an imbalance between H₂O₂ generation and metabolism that would promote oxidative stress and damage within the cell. Such an imbalance might be due to reduced activity of the enzymes contributing to H₂O₂ elimination, such as catalase and glutathione peroxidase.^{35,42} The results support the hypothesis that deterioration of the mitochondrial function and increased oxidative damage are involved in AD pathogenesis. It is important, nonetheless, to point out that other genes linked to oxidative stress and antioxidant pathways may also affect susceptibility to developing AD, and that their genetic variability should therefore be studied.

Conflict of interest

The authors have no conflict of interest to declare.

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