Genetic Polymorphisms in Apolipoprotein E and Glutathione Peroxidase 1 Genes in the Ecuadorian Population Affected With Alzheimer's Disease

César Paz-y-Miño, MD, Caty Carrera, MD, Andrés López-Cortés, BSc, María José Muñoz, BSc, Nadia Cumbal, BSc, Bernardo Castro, BSc, Alejandro Cabrera, BSc and María Eugenia Sánchez, MSc

Abstract: *Introduction:* The main objective of this study is to determine the prevalence of apolipoprotein E (*Apo E*) and glutathione peroxidase 1 (*GPX1*) polymorphisms and their influence on the development of Alzheimer disease (AD) in the Ecuadorian population. *Methods:* The authors performed an analytic transversal case-control study. The study group (n = 39) consisted of patients with AD and dementia. The control group (n = 39) comprised elderly adults who have not been diagnosed with dementia and have the same age and education as the study group. Their inclusion period was from 2007 to 2008. Later on, after obtaining informed consent and after finishing a structural interview; the next step forward was to collect blood and extract DNA by standardized protocols. Besides, the authors performed polymerase chain reaction-restriction fragment length polymorphism technique to determine the genotype of each individual. *Results:* The authors found a positive association between ε4 and ε2 alleles of *Apo E*. The *GPX1* gene shows an association of leu allele, whereas pro allele shows a negative association. The odds ratio test shows no significant relative risk. *Conclusions: Apo E* is not a risk factor, nor a protective one for AD, whereas the leu allele of *GPX1* is a possible risk factor for the disease.

Key Indexing Terms: Alzheimer's disease; *Apo E*; *GPX1*; PCR-RFLP. **[Am J Med Sci 2010;340(5):373–377.]**

Alzheimer disease (AD) is the most common cause of dementia and represents a major public health problem. AD is a progressive, neurodegenerative, irreversible, polygenic and multifactorial disorder characterized by alterations and growing detriment of the cognitive function.[1]

The Alzheimer Disease International estimates that 35 million people around the world have the disease while 4.6 million new cases are reported every year.[2] Its prevalence in the United States is approximately 8% at the age of 65 years and 30% in individuals older than 90 years.[3] In Latin America, the prevalence of dementia varies between 2.6% and 6.5% in a population older than 65 years.[4] In 2005, it was reported that, in Ecuador, there were 170 hospital admissions because of dementia, 42% of which were caused by AD.

From the Facultad de Ciencias de la Salud (CPYM, ALC, MJM, NC, BC, AC, MES), Instituto de Investigaciones Biomédicas, Universidad de las Américas, Quito, Ecuador; Facultad de Medicina (CC), Pontificia Universidad Católica del Ecuador, Quito, Ecuador; and Facultad de Ciencias de la Vida (NC), Carrera de Ingeniería en Biotecnología. Escuela Politécnica del Ejéricto, Sangolquí, Ecuador.

Submitted January 4, 2010; accepted in revised form May 21, 2010.

This study was supported by Secretaría Nacional de Ciencia y Tecnología (SENACYT).

Correspondence: César Paz-y-Miño, MD, Facultad de Ciencias de la Salud, Instituto de Investigaciones Biomédicas, Universidad de las Américas, Av. de los Granados E12-41 y Colimes, 1er P., Quito 1712842, Ecuador (E-mail: cpazymino@udla.edu.ec).

The brains affected by the disorder are known for presenting neuritic plaques and neurofibrilar tangles placed diffusively throughout the cerebral cortex and hippocampus. In addition, the amyloid deposit can be found in arteries, venules or capillaries of the cerebral cortex.[5] The neuropathologic and biochemical changes found in the disease include the arising production of amyloid protein,[6] a process that blocks synapses, alters physiology and causes neuronal death.

The precursor amyloid protein is normally divided by α secretase, an enzyme that generates 2 soluble peptides called C83 and amyloid precursor protein (sAPP) α in response to the electric and cholinergic activity. However, if APP is cut by the BACE protease and the Y secretase, it generates the C99 and β amyloid peptides, respectively.[7] These insoluble deposits along with microglia, astrocytes, dystrophic cells and proteoglycans are known as neuritic plaques, which stop nervous synopsis, alter ionic channels and induce inflammatory response and neuronal death.[8] The β amyloid can cause mitochondrial oxidative stress, a situation that causes changes in calcium regulation,[9] leads to reduced production of adenosine triphosphate, alters the electron transport and creates superoxide. The superoxide species are turned into hydrogen peroxide that interacts with nitrous oxide, iron and copper to generate peroxynitrites and free hydroxyl radicals known for affecting homeostases of cell membranes and endoplasmic reticule membranes by stimulating high calcium inflow that ultimately renders a vulnerable neuron.[10] In the development of AD, there are many genes involved that interact with the environment, regulating the risk of showing signs of the disorder,[8] such as APP, preseniline-1, preseniline-2 and the apolypoprotein E (*Apo E*) genes.[11] One poorly studied gene is the glutathione peroxidase 1 (*GPX*) 1 gene that is involved in the removal of oxygen reactive species that prevents oxidative stress, a cause for developing the disease.

The *Apo E* gene is located on the 19q13 locus; it is composed of 4 exons, and it presents a common polymorphism in its exon 3, which is characterized by 3 different protein isoforms: ε2, ε3 and ε4 result in the substitution of cysteine residues by arginine in the 112 and 158 codons.[12] The *Apo E* gene translates into a 34-KDa glycoprotein expressed in the liver, brain, kidneys, suprarenal glands, ovaries and placenta.[12] It is released to the plasma where it transports and distributes cholesterol during growth and neuronal regeneration; although in high concentrations, the protein can be toxic and lead to neuronal death.[13] The *Apo E* ε4 is quickly joined to the β amyloid and facilitates fibrillation toward the formation of neuritic plaque; this is the reason why amyloid deposits are bigger and more numerous in the carriers of the polymorphism.[13] *Apo E* ε4 has been known as a risk gene in many populations, and it is considered as the most important susceptibility factor for AD development. The risk of devel-

oping the disease is 30% in ε4ε4 genotype carriers, a risk that increases at the age of 70 years to 45% in men and 25% in women.[14]

The *GPX1* gene is located in the 3p21 locus, the pro198leu polymorphism involves a change of thymine (T) for cytosine (C), which leads to the substitution of leucine (leu) for proline (pro), whose recessive allele leu has been linked to 70% reduction of enzyme activity.[15] *GPX1* translated into a 22-KDa tetrameric protein that is expressed in erythrocytes, liver, kidney and brain.[16,17] This enzyme is the final regulator of the metabolic pathway that degrades oxygen reactive species such as peroxide radicals, limiting its interaction with nitric oxide, copper or iron and, therefore, preventing the formation of cellular oxidants.[9] The chemical reaction requires reduced glutathione; for this reason, the system has to be intact to guarantee a proper hydroxyperoxide detoxification that meets its protective function. Moreover, its activity is strongly linked to apoptosis signals and phosphorylation of protein kinases.[18] In addition, it has been found that reduced values of the *GPX1* enzyme in patients with AD along with high levels of malondialdehyde are indicators of oxidative stress.[19] The purpose of the study is to determine the prevalence of polymorphisms of the *Apo E* and *GPX1* genes and their influence on the development of the AD in individuals from the Ecuadorian population.

MATERIALS AND METHODS

Biological Samples

An analytic, transversal study of case control was performed. Seventy-eight samples of peripheral blood from Ecuadorian individuals were obtained; 39 were diagnosed with AD, and 39 were healthy individuals. The analysis and blood sample collection were realized through an informed consent from intern patients from Sagrado Corazón de Jesús Psychiatric Hospital, from Nuestra Señora de Guadalupe Clinic, from geriatric homes and health centers. The clinical histories from healthy and affected individuals exclude any other disease that may cause cognitive decline.

Genotyping

DNA extraction from peripheral blood samples of affected and healthy individuals was performed using the salting-out technique with proteinase K.[20] Low DNA Mass Ladder was used to measure DNA purity and concentration (Invitrogen, Carlsbad, CA), and a spectrophotometry of the samples was used with Spectronic Genesis 2 (Milton Roy, Rochester, NY), obtaining an optimal concentration for polymerase chain reaction (PCR) of 50 ng/μL.

Referring to *Apo E* gene, a 244-bp fragment was amplified. Each PCR reaction contained 300 ng of DNA, 200 μM of deoxynucleotide triphosphates), 10 pmol of primers FW 5′-TCCAAGGAGCTGCAGGCGG-3′ and RV 5′-GCTCGCGGATGGCGCTGA-3′,[21] 1.5 mM of $MgCl_2$, 20 mM of PCR buffer 10× (200 mM Tris-HCl pH 8.4 and 500 mM KCl), 5U of Taq DNA polymerase, 10% of dimethylsulfoxide and made up to 30 μL with MilliQ water. The amplification program consisted of 5 minutes of initial denaturation at 95°C, 1 minute of denaturation at 95°C, 1 minute of annealing at 60°C, 2 minutes of elongation at 70°C for 35 cycles and 10 minutes of final elongation at 72°C. About the *GPX1* gene, a 191-bp fragment was amplified. Each PCR reaction was performed using 100 ng of DNA, 200 μM of deoxynucleotide triphosphates, 10 pmol of primers FW 5′-CTACGCAGGTACAGCCGCCGCT-3′ and RV 5′-AAGGTGTTCCTCCCTCGTAGGT-3′,[22] 1.5 mM of $MgCl_2$, 20 mM of PCR buffer 10×, 0.5U Taq DNA polymerase and made up to 20 μL with MilliQ water. Amplification program consisted of 8 minutes of initial denaturation at 94°C, 1 minute of denaturation at 94°C, 1 minute of annealing at 60°C, 1 minute of elongation at 72°C for 36 cycles and 9 minutes of final elongation at 72°C. Amplification of both fragments was realized in an MJ Research PTC 200 thermocycler (MJ-Research Inc., Watertown, MA). Amplicons were confirmed using electrophoresis in agarose gel at 2% and observed using a UV transilluminator.

Genotyping of healthy and affected individuals with AD was realized using PCR-restriction fragment length polymorphism technique. Restriction enzyme *Hha*I (New England Biolabs, Beverly, MA) was used to digest the fragment of the *Apo E* gene. In the same way, another restriction enzyme, *Apa*I, was used to digest the *GPX1* gene. Ten units per microliter of the restriction enzymes were added to 10 μL of the PCR products, 2 μL of the digestion buffer (50 mM Tris HCl, pH 7.5, 50 mM KCl, 1 mM ethylenediaminetetraacetic acid, 10 mM 3-mercaptoethanol, 200 μL/mL bovine serum albumin, 50% glycerol and 0.1% Triton X-100) and made up to 20 μL with MilliQ water. The mixture was incubated for 3 hours at 37°C. The digested PCR products from *Apo E* with *Hha*I present various fragments according to its genotype (ε2 = 91 bp and 83 bp; ε3 = 91 bp and 48 bp; ε4 = 72 bp and 48 bp); the amplicons of the *GPX1* gene were digested with *Apa*I restriction enzyme, obtaining different fragments according to the genotype of the studied individuals (pro/pro = 191 bp; pro/leu = 191 bp, 117 and 74 bp; leu/leu = 117 and 74 bp). These fragments were confirmed using electrophoresis in agarose gel at 5% and observed with a UV transilluminator.

Statistical Analysis

Obtained information of studied individuals was statistically analyzed using SPSS 11.5 (SPSS, Chicago, IL). Percentages, averages, standard deviation, maximum, minimum and variable bias are used as measurement of descriptive resume. Significant difference was determined between genotype of healthy individuals and control using χ^2 statistic test, a significance level of 0.05 with 2 *df* and Yates correction. If the table present values are lower than 5, Fisher test was used. To determine the relative risk of developing the disease, an odds ratio (OR) test was performed using a 2 × 2 contingency table.

RESULTS

Descriptive Analysis

The investigation was realized to 78 Ecuadorian individuals. Thirty-nine of them were affected with AD, and 39 were healthy individuals without clinical antecedents of dementia. The considered descriptive variables in this study were sex, age and educational level. The groups, both affected and healthy individuals, were conformed in 69% by women (n = 27) and 31% by men (n = 12). As for the age, most of the studied individuals were in the range of 75 to 79 years. Referring to the educational level, 46% of the individuals passed primary education, 41% passed secondary education and 12% took superior studies (Table 1).

Genotyping of *Apo E* and *GPX1* Genes

In Table 2, the genotypic and allelic frequencies of the *Apo E* gene are shown. The ε2ε3 genotype was present in 5.2% of the affected individuals and in 2.6% of the control individuals; the ε2ε4 genotype was present in 2.6% of the affected individuals. The ε3ε3 genotype was present with more frequency in control individuals (79.5%) than in affected individuals (61.5%), followed by ε3ε4 genotype, with 15.4% in

TABLE 1. Descriptive values of the study population

	n (%)	Minimum	Maximum	Average	SD	Bias
Affected (n = 39)						
Gender						
Female	27 (69.2)					
Male	12 (30.8)					
FHMI						
Yes	3 (7.7)					
No	36 (92.3)					
Age (yr)		57	89	78.4	6.8	−0.65
Education (yr)		1	20	8.7	4.2	0.36
Controls (n = 39)						
Gender						
Female	27 (69.2)					
Male	12 (30.8)					
FHMI						
Yes	3 (7.7)					
No	36 (92.3)					
Age (yr)		65	89	77.9	6.5	−0.2
Education (yr)		1	16	7.9	3.9	0.16

SD, standard deviation; FHMI, familiar history of mental illness.

control individuals and 25.6% in affected individuals. The ε4ε4 variable was found in 2.6% of healthy individuals and in 5.1% of affected individuals. As for the allelic frequencies of *Apo E* gene, ε3 is more common in the affected group with 76.9% and 88.5% in the control group. The ε4 allele was present in 17.94% of affected individuals and in 10.3% of the control group; meanwhile, the ε2 allele is present in 3.8% of the patients and in 1.3% of the control group.

Table 3 shows the genotypic and allelic frequencies of the *GPX1* gene. The 61.5% of the control individuals and 25.6% of the affected individuals present the pro/pro genotype; 35.9% of the cases and 25.6% of the control group present the pro/leu genotype; and 38.5% of the affected individuals and 12.8% of control individuals present the leu/leu genotype. Corresponding allelic frequencies to *GPX1* gene show 56.4% of affected individuals and 25.6% of healthy individuals for leu allele; 43.6% of the cases and 74.4% of the controls present the pro allele.

TABLE 2. Genotypic and allelic frequencies of *Apo E* gene

	Affected (%)	Health (%)	χ^{2a}	*P*	OR (95% CI)[b]
Genotypic frequency					
ε2ε3	5.2	2.6		0.58	2.58 (0.2–76.8)
ε2ε4	2.6	0			
ε3ε3	61.5	79.5		Normal genotype	
ε3ε4	25.6	15.4	1.09	0.29	2.15 (0.6–7.9)
ε4ε4	5.1	2.6		0.58[b]	2.58 (0.2–76.8)
Allelic frequency					
ε2	3.8	1.3		0.34[b]	3.45 (0.31–88.5)
ε3	76.9	88.5		Normal genotype	
ε4	17.9	10.3	1.57	0.21	2.01 (0.73–5.9)

[a] With Yates correction.
[b] Fisher test.
OR, odds ratio.

DISCUSSION

AD is a health issue that, along with the increase of elderly population, will see an increasing prevalence during upcoming years. In the United States, it is estimated that 5.1 million elderly individuals have the disease, and this number will grow to 5.7 million in the next years.[23]

There are many events involved in this pathologic process, which include abnormalities in the β amyloid physiology, oxidative stress, inflammation and neuronal destruction.[24] Because of the complexity of the illness, it is necessary to determine the agents that play a role in its development, genetic and environmental events and also the susceptibility of disorder caused by the interaction of the aforementioned factors. Agents such as education and family background have been shown to intervene in the progression of disease.[25]

TABLE 3. Genotypic and allelic frequencies of *GPX-1* gene

	Affected (%)	Healthy (%)	χ^2 yates[a]	*P*	OR (95% CI)[b]
Genotypic frequency					
Leucine/leucine	38.5	12.8	8.77	0.003	7.2 (1.8–31.1)
Proline/leucine	35.9	25.6	3.73	0.053	3.4 (0.9–11.7)
Proline/proline	25.6	61.5		Normal genotype	
Allelic frequency					
Leucine[c]	56.4	25.6	22.8	0.000	5.05 (2.5–10.3)
Proline[d]	43.6	74.4	14.02	0.000	0.27 (0.1–0.6)

[a] With Yates correction.
OR, odds ratio.

The first link between *Apo E* ε4 and AD was reported by Corder et al[11] and Strittmatter and Roses.[26] *Apo E* ε4 is considered as the most important risk factor that has been studied so far, whereas the ε2 allele can be considered as a protective factor.[26]

The brain demands high quantities of oxygen and is provided by lipids that turn it into a target of reactive species that are product of cellular metabolism. These species interact with DNA, causing mutations, meaning that an adequate function of detoxifying enzymes such as *GPX1* is key to avoid the neurodegeneration and alteration of DNA.[27]

Both genes directly act in the neurotoxicity process. *Apo E* acts as a direct neuron aggressor when found in high concentrations, and it also regulates toxic amyloid deposits, whereas a mistaken activity of *GPX1* leaves the neuron vulnerable and prone to apoptosis.[28]

This study was performed with 78 individuals divided into 2 groups, healthy and affected, who came from different provinces of the country. Both groups were homogenous in variables, such as gender, age and education.

This is the first study that investigates AD-associated genotypes in Ecuador. The first genotype ε3ε3 was the most frequent in both groups and follows a normal distribution.[29] Meanwhile, the ε2ε2 genotype is not present in any individual of the study, a result that goes along with the findings in American indigenous and mixed-race populations where the genotype has also been absent.[30]

The distribution of the ε3 allele is similar to those reported in Caucasian population and Hispanic population of Colombia and Brazil, with allelic frequencies of 71% to 78% in affected cases and 85% in controls.[31,32]

The frequencies of the ε4 allele are the lowest reported in Latin America and Caucasian-Nordic populations where its incidence is of 44% in affected group and 17% in controls.[33] Moreover, a similar study in Spanish population reveals frequencies of 19% to 42% in affected group and 10% in controls.[21] This very own allele presents high frequencies in affected Afro-American (33%–51%) and 17% in controls. In addition, the Asian population reports 29% to 40% frequencies in affected individuals and 10% in controls.[34] These findings may be because of the Amerindian contribution in the Ecuadorian population.

In the analysis of the *Apo E* ε4 allele, there is a noticeable difference between the affected and control groups even though there is no significant difference. Many investigations performed in populations from around the world have shown that people with AD present high frequencies of the ε4 allele in the *Apo E* gene. It is very likely that the no significant difference related to the presence of the *Apo E* ε4 allele in the Ecuadorian population is because of the limited sample number processed and analyzed.

The risk of developing AD is of 2.58 times more according to the ε4ε4 genotype, and 2.01 times more for the ε4 allele and 2.29 times in presence of the ε4 allele. These findings are similar to those from other populations classified and analyzed as Caucasian, Hispanic, African and Spanish.[11,35]

The ε2 allele is present in individuals with ε2ε3 genotype. The OR indicates that, by not being a modulation of the disease, it is not linked to this allele.

As far as the genotypes found in the *GPX1* gene, there was a statistical significant difference and a link between the presence of the leu allele and the population with AD. The leu/leu and pro/leu genotypes provide a relative risk of 7.2 and 3.4, respectively. Regardless of the significant values obtained in the χ^2 test, the OR test showed values whose confidence intervals are not enough to establish a relative risk between the presence of the leu allele and the development of AD. One of the reasons for these results might be the limited number of affected and control individuals who were analyzed. The pro allele presented a significant OR of 0.27, meaning that it is a protective factor for the disease.

Each genotype modulates the function, metabolism and enzyme action of *GPX*. Therefore, the leu/leu genotype has shown to decrease enzyme activity by 70%,[15] limits the detoxifying ability that favors early neuronal death and intervenes in the development of AD. It is important to mention that the data obtained in this research comprise a starting point for future investigation in the fields of enzymatic activity and genetic expression of both the *GPX1* and *Apo E* genes. As far as the *GPX1* gene goes, it is important to correlate the *GPX* levels of enzymatic activity of the individuals with leu/leu genotype and those of the individuals with other genotypes, thereby determining the influence of the polymorphic variants with regard to the overexpression or reduced enzyme activity in the Ecuadorian population.

The geographic and ethnic heterogeneity in Ecuador has been further proved by the findings of molecular epidemiology and polymorphic incidence of the *FQ*, *BCR-ABL*, *hMSH2* and *CYP1A1* genes.[36–38]

ACKNOWLEDGMENT

We thank the hospitals, clinics, foundations, health centers and geriatric homes for the support in obtaining peripheral blood samples of individuals with Alzheimer disease.

REFERENCES

1. **Bird T.** Genetic aspects of Alzheimer disease. Genet Med 2008;10: 231–9.
2. **Ferri CP, Sousa R, Albasese E, et al.** World Alzheimer report 2009—executive summary. Pince M, Jackson J, editors. London (UK): Alzheimer's Disease International; 2009. p. 1–22.
3. **Brookmeyer R, Gray S, Kawas C.** Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. Am J Public Health 1998;88:1337–42.
4. **Rodriguez L, Ferri C, Guerra M, et al.** Prevalence of dementia in Latin America, India and China: a population-based cross sectional survey. Lancet 2008;372:430–2.
5. **Selkoe D.** Alzheimer's disease: genes, proteins and therapy. Physiol Rev 2001;81:741–66.
6. **Bales KR, Dodart JC, DeMattos RB, et al.** Apolipoprotein E, amyloid, and Alzheimer disease. Mol Interv 2002;2:363–75.
7. **García-Ruíz PJ, Echeverría A, García A, et al.** Nuevas opciones terapéuticas en la enfermedad de alzheimer [in Spanish]. Rev Neurol 2006;42:478–81.
8. **Thomas P, Fenech M.** A review of genome mutation and Alzheimer's disease. Mutagenesis 2006;22:15–33.
9. **Reddy P.** Amyloid precursor protein-mediated free radicals and oxidative damage: implications for the development and progression of Alzheimer's disease. J Neurochem 2006;96:1–13.
10. **Mattson M.** Pathways towards and away from Alzheimer's Disease. Nature 2004;30:631–9.
11. **Corder E, Saunders A, Strittmatter W, et al.** Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921–3.
12. **Paik Y, Chang DJ, Reardon CA, et al.** Nucleotide sequence and structural of the human apolipoprotein E gene. Proc Natl Acad Sci U S A 1985;82:3445–9.

13. **Huang Y.** Apolipoprotein E and Alzheimer's disease. Neurology 2006; 66:s79–85.
14. **Bird T.** Alzheimer disease overview. Gene Rev 2007;1–19.
15. **Hamanishi T, Furuta H, Kato H, et al.** Functional variants in the glutathione peroxidase-1 (*GPX-1*) gene are associated with increased intima-media thickness of carotid arteries and risk of macromolecular disease in Japanese type 2 diabetic patients. Diabetes 2004;53:2455–60.
16. **Cisneros E, Pupo J, Céspedes E.** [Enzimas que participan como barreras fisiológicas para eliminar los radicales libres: III Glutatión peroxidasa]. Rev Cubana de Invest Biomed 1997;16:10–5.
17. **Power J, Blumbergs P.** Cellular glutathione peroxidase in human brain: cellular distribution and its potential role in the degradation of Lewy bodies in Parkinson's disease and dementia with Lewy bodies. Acta Neuropathol 2009;117:63–73.
18. **Lei X, Cheng W, McClung J.** Metabolic regulation and function of glutathione peroxidase 1. Annu Rev Nutr 2007;27:41–61.
19. **Casado A, López-Fernandez M, Casado M, et al.** Lipid peroxidation and antioxidant enzyme activities in vascular and Alzheimer disease. Neurochem Res 2008;33:450–8.
20. **Sambrook J, Fritsch E, Maniatis T.** Molecular cloning: a laboratory manual, 2nd ed. New York (NY): Cold Spring Harbor Laboratory Press; 1989.
21. **Beyer K.** [Caracterización genética de la enfermedad de Alzheimer: estudio poblacional]. Barcelona (Spain): Universidad Autónoma de Barcelona; 2002.
22. **Ichimura Y, Tomonori H, Norihiki T, et al.** Increased risk of bladder cancer associated with a glutathione peroxidase 1 codon 198 variant. T Urol 2004;172:728–32.
23. **Hebert L, Scherr P, Bienias J, et al.** Alzheimer disease in the US population. Arch Neurol 2003;60:1119–22.
24. **Felican O, Sandon T.** The neurobiology and pharmacotherapy of Alzheimer's disease. J Neuropsychiatry Clin Neurosci 1999;11:19–31.
25. **Huang W, Qiu C, Strauss E, et al.** APOE genotype, family history of dementia and Alzheimer disease risk. Arch Neurol 2004;61:1930–4.
26. **Strittmater W, Roses A.** Apolipoprotein E and Alzheimer disease. Proc Natl Acad Sci U S A 1995;92:4725–7.
27. **Rao K.** Free radicals induced oxidative damage to DNA: relation to brain aging and neurological disorders. Indian J Biochem Biophys 2009;46:9–15.
28. **Jimenez-Jimenez F, Alonso-Navarro H, Ayuso-Peralta L, et al.** [Estrés oxidativo y enfermedad de Alzheimer]. Rev Neurol 2006;42: 419–27.
29. **Farrer L, Cupples L, Haines J, et al.** Effects of age, sex and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis APOE and Alzheimer disease metaanalysis consortium. JAMA 1997;278:1349–56.
30. **Gamboa R, Hernández-Pacheco G, Hesiquio R, et al.** Apolipoprotein E polymorphism in the Indian and Mestizo population of Mexico. Hum Biol 2000;72:975–81.
31. **Jacquier M, Arango D, Villareal E, et al.** *Apo E* ε4 and Alzheimer's disease. Positive association in a Colombian clinical series and review of Latin American studies. Arq de Neuropsiquiatr 2001;59:1911–7.
32. **Almeida O.** Apolipoprotein E ε4 and Alzheimer's disease in Sao Paulo-Brasil. Arq Neuropsiquiatr 1997;55:1–7.
33. **Helisalmi S.** Molecular genetics of Alzheimer's disease. With special emphasis on presenilin, amyloid beta precursor protein and apolipoprotein E genes. Series of reports Kuopio University Hospital. Kuopio University Hospital; 1998;44.
34. **Nunomura A, Chiba S, Eto Masaaki, et al.** Apolipoprotein E polymorphism and susceptibility to early and late onset sporadic Alzheimer's disease in Hokkaido, the northern part of Japan. Neurosc Lett 1996;206:17–20.
35. **Bufill E, Bartés A, Moral A, et al.** [Factores genéticos y ambientales que pueden influir en la forma senil de la enfermedad de Alzheimer: estudio de casos y controles anidado]. Neurología 2009;42:108–12.
36. **Paz-y-Miño C, Burgos R, Morillo S, et al.** BCR-ABL rearrangement frequencies in CML an ALL in Ecuador, South America. Cancer Genet Cytogenet 1998;132:65–7.
37. **Paz-y-Miño C, Perez J, Fiallo B, et al.** A polymorphism in the hMSH2 gene (gIVS12-6T>C) associated with non Hodgkin lymphomas. Cancer Genet Cytogenet 2001;133:29–33.
38. **Paz-y-Miño C, Arévalo M, Sánchez M, et al.** Chromosome and DNA damage analysis in individuals occupationally exposed to pesticides with relation to genetic polymorphism for CYP1A1 gene in Ecuador. Mutat Res 2004;562:77–89.