ARTÍCULO ORIGINAL

Diabetes mellitus in patients with Alzheimer's disease: clinical description and correlation with the *APOE* genotype in a sample population from the province of Antioquia, Colombia

Luz E. Botero[1,2*], Andrés E. Toro[1*], Alber J. Patiño[1*], Guillermo Salazar[1*], Juan C. Rodríguez[1*], Juan C. Suárez-Escudero[1,3], Gustavo A. Alarcón[1], Ana Corcimaru[2,4,5], Cristina Osorio[2,4,6], Joseph S. Y. Jeong[2,4,5], and Oscar Alzate[1,2,4,5,6]

[1] Escuela de Ciencias de la Salud, Universidad Pontificia Bolivariana, Medellín, Colombia
[2] Grupo de Biología Estructural y Proteómica Group, Universidad Pontificia Bolivariana, Medellín, Colombia
[3] Fundación Instituto Neurológico de Colombia (INDEC), Medellín, Colombia
[4] Systems Proteomics Center, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, USA
[5] Department of Cell and Developmental Biology, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, USA
[6] Program in Molecular Biology and Biotechnology, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, USA
* These authors contributed equally to this work

Introduction. Alzheimer's disease is a multifactorial disease affecting approximately twenty million people worldwide. Numerous variables are associated with increased risk of developing this severe neurological disorder. Among the risk factors, diabetes mellitus, and the ε4 isoform of the *APOE* gene have been amply demonstrated as increasing the risk of developing this disease.

Objective. To determine if a correlation exists between *APOE* genotype, diabetes mellitus and Alzheimer's disease.

Materials and methods. Clinical studies were carried out by surveying the clinical histories in a group of patients in the province of Antioquia, Colombia. Forty-three Alzheimer's patients were compared with 43 control subjects, paired by age and gender. Commercially available methods were used to determine whether the patients had diabetes, and restriction enzyme-based genotyping was used to determine the *APOE* genotypes.

Results. The most common non-neurological comorbidities were: arterial hypertension, acute myocardial infarction, chronic obstructive pulmonary disease and hypothyroidism. From the many variables investigated, two were conclusive: (1) the presence of Alzheimer's disease was higher in patients with diabetes mellitus, and (2) no correlation between late-onset sporadic Alzheimer's disease and *APOE* was found in the target population.

Conclusions. To detect any association with the *APOE* genotype, a study involving much a larger population samples must be undertaken.

Keywords: Alzheimer's disease, diabetes mellitus, apolipoprotein E, dementia; pulmonary disease, chronic obstructive.

Diabetes mellitus en pacientes con enfermedad de Alzheimer: descripción clínica y correlación con el genotipo *APOE* en una muestra de población del departamento de Antioquia, Colombia

Introducción. La enfermedad de Alzheimer es compleja y afecta, aproximadamente, a 20 millones de personas en todo el mundo. Muchas variables parecen aumentar el riesgo de desarrollar esta alteración neurológica. Entre los factores de riesgo, se ha demostrado ampliamente que la diabetes

mellitus y la isoforma ε4 del gen *APOE* tienen incidencia positiva en el desarrollo de la enfermedad.
Se reporta un estudio en el cual se investigó la posible correlación entre *APOE*, diabetes mellitus y la enfermedad de Alzheimer, en un grupo específico de pacientes del departamento de Antioquia, Colombia.

Objetivo. Determinar si existe una correlación entre *APOE*, diabetes mellitus y la enfermedad de Alzheimer, en un grupo de pacientes de Antioquia, Colombia.

Materiales y métodos. Se buscaron y analizaron las historias clínicas de los pacientes con diagnóstico de enfermedad de Alzheimer. Se seleccionaron aquellos que cumplían los criterios de inclusión. Se utilizaron métodos comercialmente disponibles para confirmar la presencia de diabetes mellitus. La genotipificación de *APOE* se hizo con un método basado en la PCR y la digestión con enzimas de restricción, en muestras de todos los participantes en el estudio.

Resultados. En este estudio se analizan 43 casos de enfermedad de Alzheimer y 43 individuos sanos controles, pareados por edad y sexo. Las enfermedades concomitantes no neurológicas más comunes fueron: hipertensión arterial, infarto agudo del miocardio, enfermedad pulmonar obstructiva crónica e hipotiroidismo.

Conclusiones. De las diferentes variables investigadas, dos arrojaron resultados concluyentes: i) la presencia de la enfermedad de Alzheimer es más frecuente en pacientes con diabetes mellitus, y 2) no se encontró correlación entre la enfermedad de Alzheimer de inicio tardío esporádico y el genotipo de *APOE*. Es importante indicar que debe llevarse a cabo un estudio con un tamaño de población mayor, para determinar cualquier posible correlación o inferencia con el genotipo de *APOE*.

Palabras clave: enfermedad de Alzheimer, diabetes mellitus, apolipoproteína E, demencia, enfermedad pulmonar obstructiva crónica.

Alzheimer's disease (AD) is the most common type of dementia affecting memory, problem solving, learning, behavior and the ability to perform everyday activities. It is a syndrome that can be caused by a number of progressive disorders, most of which are currently the subject of intensive research. In the 2010 World Alzheimer Report (http://www.alz.co.uk/research/files/WorldAlzheimer Report2010Executive Summary.pdf), Alzheimer's Disease International estimated that approximately 36 million people are living with dementia worldwide. Based on this report, nearly two-thirds of the affected population live in low- and middle-income countries, where the "sharpest increases in affected patients are expected to occur". People with AD, their families and friends are affected on personal, emotional, financial and social levels. Dementia has already affected significantly every health care system in the world (1,2).

AD accounts for 50-70% of dementias (3). AD patients are mainly characterized by a progressive decline in cognitive function concomitant with the appearance of abnormal deposits of the β-amyloid peptide (Aβ) called amyloid plaques

(4), and neurofibrillary tangles (NFT) composed of aggregates of the protein *tau*. Other characteristics include synaptic dysfunction (5), decreased cerebral blood flow to the posterior portion of the left cingulate cortex (6), and cerebral endothelial dysfunction (7). Combinations of these and a variety of other factors may lead to neurodegeneration (8). Clinically, AD is defined by the presence of multiple cognitive alterations--characteristically amnesia and one or more of the following disturbances: aphasia, apraxia, agnosia, and alterations of normal executive functioning with progressive neurologic decline (9).

Until now, definitive diagnosis of AD has been by *postmortem* neuropathological analysis, based on the following observations: diffuse atrophy (mainly in the hippocampus, Sommer's CA1 sector), decreased number of neurons in the basal nucleus of Meynert, hyperphosphorylated neurofibrillary tangles (hyperphosphorylated *tau* protein), senile plaques, granulovacuoles (tubulin) and Hirano bodies (actin) (10). These histopathological features are commonly found in the hippocampus, temporal cortex, and basal nucleus of Meynert (lateral *septum*). In a normally-aging brain, low levels of these alterations are expected, compared to the large amounts in an AD-affected brain (10,11).

The etiology of AD has not yet been clearly established (12). Both heredity and environmental conditions may play important roles in the onset and progression of AD, as suggested by the existence of different clinical presentations (*i.e.*, early-onset

Corresponding author:
Oscar Alzate, Ph.D., 438A Taylor Hall, Mason Farm Road, Department of Cell and Developmental Biology, CB# 7090, Chapel Hill, NC 27599, USA
Phone: (919) 962 3698; fax: (919) 966 1856
alzate@med.unc.edu

AD (EOAD, divided into early-onset familial or eFAD, and early-onset sporadic) and late-onset AD (LOAD, also divided into late-onset familial and late-onset sporadic)) (12,13). Age is the most significant risk factor for AD (14). Autosomal dominant EOAD (younger than 65 years of age) has been associated with point mutations on the *APP* (amyloid precursor protein) or the *PSN1*, *PSN2* (presenilin) genes. However, this form of the disease accounts for less than 5% of the total number of AD patients (12). The *APOE* gene, which codes coding for the apolipoprotein E (apoE), has been associated with increased risk of developing LOAD –the most common form of the disease (15).

Diabetes mellitus (DM) is characterized by chronic systemic hyperglycemia in which defects in insulin secretion, peripheral resistance to insulin and increased production of glucose alter the metabolism of carbohydrates, fats and proteins (16). Chronic hyperglycemia in diabetes has been associated with long-term damage of the eyes, kidneys, heart, nervous system and blood vessels (17,18). Four main categories of DM have been identified: Type 1 DM (DM1), Type 2 DM (DM2), other specific types and gestational diabetes (16).

Interestingly, the prevalence of DM1 and DM2 is increasing worldwide (19). DM increases with age, and, in individuals older than 60 years of age, the prevalence of DM has been estimated to be as high as 21% (20). DM prevalence is similar among men and women throughout most age ranges (10.5% and 8.8% in individuals >20 years of age) except for people older than sixty years of age, in which men present a slightly greater prevalence (21). More than 15 million people have DM in Latin America, a number that is expected to increase (22). The diagnosis of DM2 is usually late and often results from the emergence of various complications including retinopathy (ranging from 16-21%), nephropathy (12-23%) and neuropathy (25-40%), with the result that about 30-50% of people suffering from DM2 are unaware of their condition (23).

A recent estimate suggests that diabetes is the fifth leading cause of death worldwide and is responsible for almost 3 million deaths annually (21). DM is included in the list of chronic non-transmissible diseases. The magnitude of this WHO epidemiological category is such that it accounts for 60% of all deaths worldwide, surpassing by a high margin the group of infectious diseases, including HIV/AIDS, TB and malaria.

DM is another risk for AD (24). The relationship between DM and AD has been found to be particularly strong in patients with the ε4 allele of *APOE* (*APOE4*) (25), and DM has been proposed to be associated with sporadic and familial LOAD (26-28).

DM2 and the *APOE4* allele have been found to be associated with an increased amount of amyloid plaques in the hippocampus, increased amount of NFT and increased cerebral amyloid angiopathy (8,29,30). ApoE is a circulating lipoprotein, which, by binding the low density lipoprotein (LDL) receptor, promotes the intracellular metabolism of lipids (31-33). This protein is vital for the normal metabolism of triglyceride-rich lipoproteins (32). The *APOE* gene is polymorphic, with three common alleles (ε2, ε3, and ε4); these are linked to different lipid metabolic profiles, but not showing a particular distribution in DM2 (34).

The *APOE4* genotype increases the risk of developing AD (15). ApoE is important for maintenance and repair of neurons and apoE3 is more efficient than apoE4 in this regard (33). Histopathological data indicate that the expression of the insulin-degrading enzyme in the hippocampus is substantially reduced relative to controls, in particular in AD patients with the *APOE4* allele (35).

The genotype displaying the highest risk for developing sporadic LOAD is *APOE4/4* (15). The *APOE3/4* genotype is associated with a medium-to-high risk for AD. *APOE3/3* genotype has a risk factor equal to the general population. *APOE2/3* genotype is correlated with a lower risk for developing AD. Those with *APOE2/4*, have a risk equivalent to *APOE3/3*, *i.e.*, similar to the rest of the population (36). In the general population the frequency of the ε3 allele is 78% and of the ε4 allele is 13%. In patients with LOAD, the ε4 allele frequency is 52%. In fact, homozygous *APOE4/4* people present almost 8 times higher risk of developing AD compared to homozygous *APOE3/3* people; although homozygousity for ε4 does not necessarily result in developing the disease (15,36). Among individuals from families with LOAD without copies of ε4, only 20% develop AD at around 75 years of age, compared to 90% of individuals with two copies of the ε4 allele (37).

In Colombia, the frequencies of the ε2, ε3, and ε4 alleles are similar to those reported in other countries. In a study conducted in 2006 in Bogotá involving residents between 18 and 106 years of age, 75% of the population were found with the *APOE3/3* genotype, 15% were of the *APOE3/4*

genotype, 8.2% were *APOE2/3*, 1.4% were of the *APOE2/4* genotype, and 0.7% of the population were *APOE2/2* or *APOE4/4* (38). Similar results were found by another study conducted in 2007 involving 1,567 individuals from the Central and East parts of the country (39).

The glycemic dysregulation found in DM has been associated with brain structural damage, including damage in large and small vessels (40). Hyperglycemia can affect neural viability by increasing oxidative stress and formation of advanced glycation end-products (AGEs) (41). It is now considered that the AGEs play an important role in the pathophysiology of various neurological diseases *via* several mechanisms: (1) by modifying extracellular structural proteins; (2) by triggering intracellular processes that bind to extracellular receptors activating signal cascades; and (3) by modifying intracellular proteins. In diabetic patients, the accumulation of AGEs leads to endothelial dysfunction among other important structural changes (41-43).

Hyperglycemia also has a direct effect on neural calcium balance in the hippocampus resulting in cell damage. It may also produce hyperosmolarity and increase in vasopressin levels, eventually leading to hypothalamic neuronal degeneration (40,44). Altered glucose metabolism may result in decreased acetylcholine production, synaptic failure, and cholinergic neuron degeneration in the hippocampus (45,46). The appearance of atherosclerosis in cerebral arteries is also associated with DM, resulting in decreased cerebral blood flow and a disturbance in vascular reactivity causing hypoxic and ischemic states, which are important pathological conditions that can cause multiple vascular dementias (6,47,48). Biessels *et al.* (49) concluded that DM increases the risk of atherosclerosis and stroke, (*i.e.*, something that produces a cerebral vascular disease, mediated by glucose toxicity and microvascular abnormalities), both of which lead to accelerated brain aging (49,50).

The accelerated formation of AGEs has been found to correlate with an increase in Aβ deposition, greater number of plaques, neurofibrillary tangles and elevated *tau* protein in the hippocampus and the cerebral cortex. Furthermore, accelerated AGEs formation is closely related to increase in oxidative stress (51). Insulin and glucose dysregulation can also alter amyloid metabolism by changing the insulin/insulin receptor interaction (52) and by the formation of AGEs (53).

DM is another risk for AD, although the molecular pathways connecting DM and AD are unknown (24). The fourth and fifth stages (known as *The Honolulu Asia Aging Study*) of the so-called "*Honolulu Heart Program*" were designed to investigate risk factors associated with neurodegenerative disorders and aging. This research program also presented substantial evidence that diabetes is a major risk factor for the development of both vascular dementia and AD (25). It was also found that the association was particularly stronger in *APOE* ε4 carriers (25). Similar results were also obtained by the "*The Cardiovascular Health Study Cognition*", a cohort study carried out from 1992 to 2000 (54).

Based on these previous publications in which DM2 and the *APOE* genotype have been found as risk factors for AD, the current study was designed to determine if a correlation was present among these variables, in a cohort of patients from the Aburrá Valley (*province* of Antioquia, Colombia), who had been diagnosed with AD. Despite using a small population (43 cases and 43 controls), a statistically significant correlation was found between DM2 and AD. All patients and controls, selected at random for this study, were either *APOE*3/3 or *APOE* 2/3, with no ε4 alleles present. Clearly, an additional future study including a larger randomized sample is necessary before a correlation can be inferred between the *APOE* genotype and AD.

Materials and methods

The study design was a descriptive case-control study. The study population was composed of individuals residing in the towns of Medellín, Envigado, Itagüí, La Estrella, Bello, and Copacabana (Antioquia Province, Colombia). The population sample (n=86) was divided into two groups: individuals with diagnosis of AD who met the DSM-IVR criteria for AD diagnosis (n=43) and control subjects who did not meet a single DSM-IVR criteria for AD (n=43).

AD patients were selected according to the following characteristics: age ≥ 65 years of age regardless of sex, diagnosed with sporadic LOAD by a neurologist, meeting the DSM-IVR clinical criteria for its diagnosis, and having no relatives with an AD diagnosis (thus selecting only for sporadic LOAD) or any other type of dementia. All patients and family members (guardian or caretaker) as well as the control group population participated voluntarily and were recruited for this study following the regulations of the ethics committee of the "Universidad Pontificia Bolivariana" and from

the ethics and research committee of the Instituto Neurológico de Colombia (INDEC).

In order to validate the diagnosis of AD, the following pathological conditions were ruled out: stroke, Parkinson's disease, Huntington's chorea, chronic subdural hematoma, normal-pressure hydrocephalus, cerebral neoplasm, depressive pseudo dementia, hypothyroidism, folic acid deficiency, vitamin B12 deficiency, neurosyphilis, HIV/AIDS, TB, Wilson's disease, cysticercosis, uremic encephalopathy, hepatic encephalopathy, autoimmune processes, and chronic sleep apnea. This was done by analyzing the medical history of the patients (who have a long term follow-up from the INDEC) and retrospective corroboration with their families at the time of the home visit.

The control group (healthy individuals) consisted of persons older than 65 years of age from both genders, without medical history of dementia syndrome (9) or any other neurodegenerative disease. These individuals did not meet any of the DSM-IVR criteria for AD, had no history of stroke, head injury, or neurosurgery at any point in their lives, and had no family history of AD.

In addition, all individuals included in the study were evaluated for DM. INDEC patients and control subjects were evaluated according to their clinical history and by validation of the condition with laboratory tests using methods commercially available. Individuals who were on steroids over a 3-month period previous to this study, or who had any glycemic alterations resulting from steroids intake, were not included in this study.

Selection of individuals

The inclusion of subjects in the study was made on the basis of a database from the INDEC that included 779 medical records of patients with diagnosis of dementia syndrome in the Neurology Outpatient service, from January 2008 to July 2009. Four hundred seventeen patients, whose
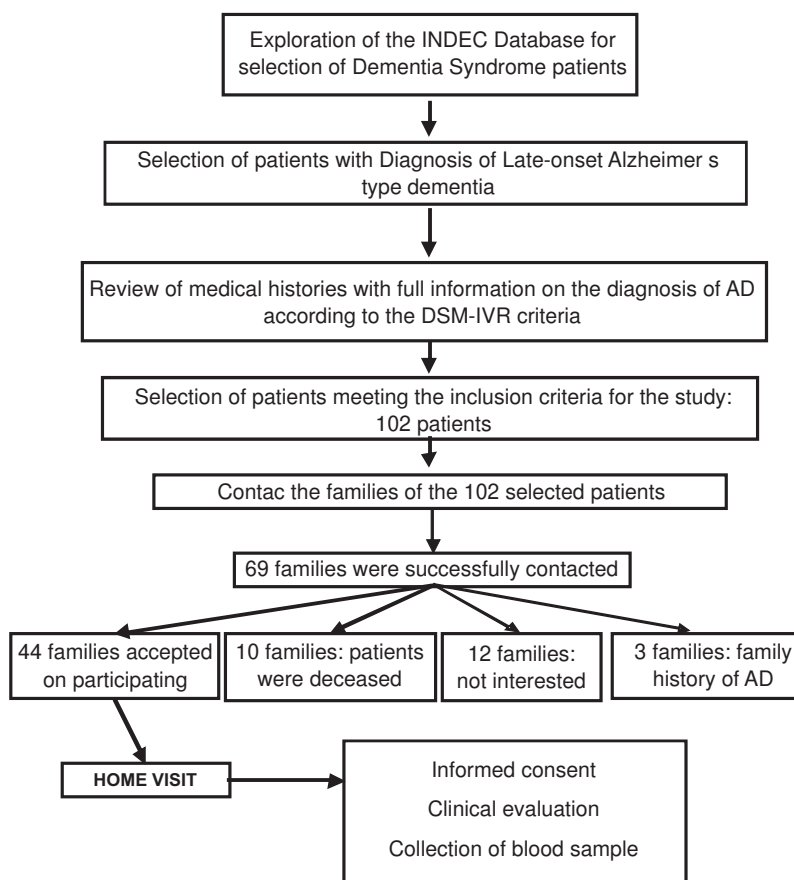

Figure 1. Experimental design for patient selection. This flowchart explains the selection (and partial results) of medical histories from the database in the INDEC for the recruitment of patients.

medical histories documented the clinical diagnosis of LOAD, were pre-selected (ICD-10 code: G30.1). From these, 190 were selected through meticulous and detailed reading of the patient's diagnostic records. Of these, 102 met the selection criteria. Following strict regulations of the INDEC protecting the privacy of the patients and their families, families of 69 patients were contacted, and 44 agreed to participate in the study–which included a home visit with each family. This experimental design is presented in figure 1.

The privacy of the participants was protected by dividing the research team into two groups. One group consisted of physicians and nurses, who interacted with the patients and their families, whereas the other team consisted of scientists performing molecular studies. At no point during this investigation did the groups exchange personal information. During the home visit, a physician from the INDEC provided a physical examination including a neurological exam to corroborate the data recorded on the medical histories (internal validity) and filled out a form with the patient's clinic-epidemiological survey. This step ascertained that the patient met the DSM-IVR diagnostic criteria for AD as already stated by the medical history. This meeting was followed by blood sampling (as described below). One patient was withdrawn from the group because it was confirmed that there was AD history in the family.

During the home visits healthy individuals were recruited from the community and were paired with AD patients according to gender and age (± 5 years). Control subjects met the pre-established inclusion and exclusion criteria and were visited by the group of researchers to their respective domiciles. At this point the clinic-epidemiological survey form was filled out, followed by blood sampling for laboratory testing.

Clinic-epidemiological survey

Since each medical history documented the diagnosis of AD, a complete anamnesis was conducted in order to confirm the information found in the medical history from the database. Therefore, each individual underwent a physical examination to determine the individual's arterial pressure, weight, height, abdominal circumference and body mass index (BMI). Individuals also underwent a complete neurological examination, verifying the health status of both AD patients and control subjects and whether they met the DSM-IVR criteria for AD.

Blood sampling, laboratory testing and DNA extraction

Approximately 10 mL of peripheral blood were taken from each individual by venipuncture (it was made sure that each patient had been fasting), using vacuum tubes (BD Vacutainer, Becton Dickinson and Co., BD&C, Franklin Lakes, NJ, USA), and 21G x 1½" needles (BD VacutainerPrecisionGlide, Multiple Sample Needle, BD&C). A portion of the blood (5mL) was collected in tubes with EDTA (BD Vacutainer K2 EDTA, Plus Blood Collection Tubes, BD&C) for extracting leukocytes which was the source of DNA for *APOE* genotyping. The rest of each sample (5 mL) was collected in reactant- and preservative-free tubes (BD Vacutainer Serum Blood Collection Tubes, BD&C) for blood chemistry analysis. Blood chemistry tests were performed at the clinical laboratory of the "Hospital Universitario Bolivariano" (University Hospital of the Universidad Pontificia Bolivariana). Fasting glucose levels, lipid profiling (total cholesterol, TC; low density lipoprotein, LDL; high density lipoprotein, HDL; very low density lipoprotein, VLDL; and atherogenic index; TC/HDL) were determined using standard procedures.

For DNA extraction, all chemicals were from Bio-Rad (Hercules, California, USA) or Sigma (St. Louis, Missouri, USA). Genomic DNA was extracted from 5 mL of peripheral blood, anti-coagulated in EDTA following the extraction method described by Miller (55) with some modifications. The DNA pellet was allowed to dry and was resuspended in 1 mL of sterile type I DNAase-free H_2O and stored at −20ºC until use. For genotyping, DNA samples were quantified and its quality was evaluated using Thermo Scientific NanoDrop 2000c by reading the absorbance of 1 µL of sample at 260nm. The 260/280 absorbance ratio was used to assess the purity of DNA. A ratio of ~1.8 was accepted as "pure" for DNA.

Genotyping of *APOE* genes was carried out with PCR-based restriction fragment length polymorphism (RFLP) as described by Hixson and Vernier (56) with some modifications. gDNA from human blood samples was amplified with primers (*APOE*-forward: TAAGCTTGGCACGGCTGTCCAAGGA; *APOE*-reverse:ACAGAATTCGCCCCGGCCTGGTACACT GCC) to obtain 244bp amplicons. Fifteen microliters of 2X PCR master mix (Fermentas, Glen Burnie, MD, USA), 300 ng of gDNA template, 1.5 µl of 10 µM of each primer and 2 µl of DMSO were mixed to 30 µl reaction. Each reaction mixture was denatured at 94ºC for 4 min and 35 cycles of amplification were performed with steps of annealing at 60ºC

for one min, extension at 72°C for two min and denaturation at 94°C for 30 seconds, followed by an extra extension step at 72°C for three min at the end of the cycles. Ten microliters of PCR product was digested in 30 µl reaction for 3 hours with 5 units of *HhaI* (New England Biolabs (NEB), Ipswich, MA, USA) with 20 µl of 1X buffer 4 (NEB); 5 µl of each reaction mixture was loaded on an 8% polyacrylamide gel in 1X TBE buffer and run for 3 hours under constant voltage of 10V/1cm. The polyacrylamide gels were stained with ethidium bromide and visualized by UV illumination. As described by Hixson and Vernier (56), *HhaI* gives different RFLP patterns because of different nucleotide sequences at aminoacid numbers 112 and 158 of ApoE2, ApoE3 and ApoE4 (figure 2).

Ethical considerations

The ethical approval for the execution of this study was obtained from the ethics committee of the "Universidad Pontificia Bolivariana" and from the ethics and research committee of the INDEC.

Access and extraction of data from the INDEC database, as well as the contact with families, was done with previous authorization from the Ethics and Research Committee of the INDEC. Information about the study, including its objectives and methods, was provided by telephone to the families or caretakers of the patients. The home visit was agreed to by the families, or directly with the control subjects. On each visit, a written informed consent was obtained from families or caretakers who signed it voluntarily, and all questions concerning the study were adequately addressed. Protecting the principles of autonomy, respect for the dignity of individuals and beneficence were always a top priority. All the information obtained during the study was confidential. Individuals were assigned with a code in order to protect their identities.

Statistical analysis

The information was obtained and recorded on the clinic-epidemiological survey form, then transferred into a database created with Microsoft Excel®. Central tendency and non-parametric measures were calculated (χ^2, confidence intervals (CI), relative risk (RR)). Statistically significant data were interpreted with a *p*-value < 0.05 and their respective confidence intervals.

Results

Forty-three AD cases and 43 control subjects were paired according to their age and gender. The
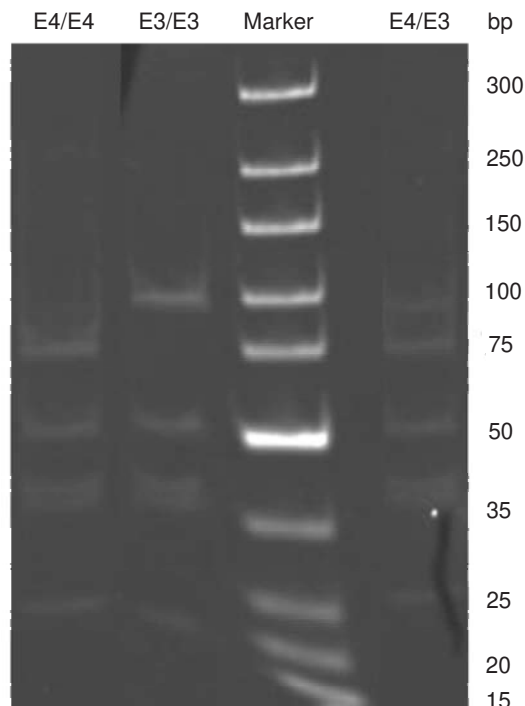

Figure 2. Genotyping for *APOE*. Five microliters of RFLP products of each reaction mixture was run in 8% polyacrylamide gel in 1X TBE buffer for 3 hours. The polyacrylamide gels were stained with ethidium bromide and visualized by UV illumination. Lane 1: *APOE4/4* homozygous, Lane 2: *APOE3/3* homozygous, Lane 4: *APOE4/3* heterozygous, Lane 3: Molecular Weight Standard (Bio-Rad).

percentage of females in both groups was 53.5%, with an average age for AD cases of 80.9 years (SD=6.7) and the average age for controls 81.7 years (SD=7.5).

The most common non-neurological comorbidities were as follows: arterial hypertension (53.5% for AD cases and 65.1% for control subjects), acute myocardial infarction (11.6% for AD cases and 4.7% for control subjects), chronic obstructive pulmonary disease (18.6% and 4.0%), hypothyroidism (18.6% and 11.6%), smoking (65.1% and 27.9%), alcohol consumption (39.5% and 16.3%), any form of cancer (16.3% and 7.0%), heart failure (11.6% and 7.0%) and dyslipidemia (9.3% and 16.2%). Comorbidities were determined from the medical histories of the population under study and subsequently confirmed with the families of the AD patient and by direct interview with the control subjects. The rest of the clinical diagnosis and history is presented in table 1.

When the non-neurological comorbidities in both groups were compared, significant statistical differences were present for renal failure and

smoking (this was the subject status only at the time of analysis; since no long term follow-up was conduted, no conclusions were possible as to whether or not these comorbidities are directly associated with AD or if they represent a causal relationship to AD). The most common neurological comorbidities were head trauma (11.6% and 7.0%) and epilepsy (9.3% and 2.3%), without statistically significant differences. The clinical pathology findings for neuropsychological and neurological evaluation showed statistically significant differences as shown in table 2.

All patients met the DSM-IVR criteria for the diagnosis of AD, displaying in each of them statistically significant differences compared to the control subjects, as shown in table 3. Most individuals in both groups presented normal body mass index (BMI) (62.5% for AD patients and 68.8% for control subjects). Only a quarter of the population was found to have a BMI above normal, 22% in the case group and 28.1% for the control group, as outlined in table 4.

At the time of the home visit, all participants underwent a clinical assessment and blood samples were taken for blood chemistry testing. The values determined for the variables measured are presented in table 5. No significant differences were found in either group. Regarding the presence of alterations in blood glucose levels, eleven of the AD patients (25.6%) and one of the control subjects were diagnosed with DM2 (statistical significance, χ^2 with Yates' correction = 9.68, p = 0.002). Diabetic nephropathy, two diabetic retinopathies, and one diabetic foot were diabetic complications found in the AD population. Diabetes was found 14 times more in AD patients than in control subjects (Odds Ratio 14.4, 95% CI 1.7-117.7).

Genotyping studies for *APOE* indicated that 89% of the population was of the *APOE*3/3 genotype, while the remaining 11% was of the *APOE*2/3 genotype. No ε4 allele was found. When evaluating the frequencies of the *APOE* genotypes in the group of patients and in the group of control subjects, the

Table 1. Description of non-neurologic and neurological comorbidities in the AD and control groups

Non-neurological and neurological comorbidities	% AD Cases (n=43)	% Controls (n=43)	OR (CI95% OR)		X^2	p-value
Arterial hypertension	53.5	65.1	0.61	(0.25-1.46)	1.20	0.272
Acute myocardial infarction	11.6	4.7	2.67	(0.49-14.73)	1.40	0.237
Angina pectoris	2.3	9.3	0.23	(0.02-2.16)	1.91	0.167
Heart failure	11.6	7.0	1.75	(0.39-7.85)	0.55	0.458
COPD	18.6	4.0	3.04	(0.75-12.39)	2.61	0.106
Arthritis	4.7	2.3	2.04	(0.17-23.47)	0.43	0.557
SLE	0.0	0.0	-		-	-
Hypothyroidism	18.6	11.6	1.73	(0.51-5.81)	0.81	0.366
Hyperthyroidism	0.0	0.0	-		-	-
Hepatitis B or C	0.0	0.0	-		-	-
Malnutrition	2.3	0.0	-		1.01	0.314
Renal failure	0.0	9.3	2.02	(1.63-2.51)	4.19*	0.041
Liver failure	0.0	0.0	-		-	-
Cancer	16.3	7.0	2.59	(0.62-10.78)	1.81*	0.178
Malaria	0.0	0.0	-		-	-
TB	0.0	0.0	-		-	-
HIV/AIDS	0.0	0.0	-		-	-
Cigarette smoking	65.1	27.9	4.82	(1.93-12.04)	11.95	0.001*
Alcohol consumption	39.5	16.3	3.36	(1.21-9.27)	5.78*	0.061
Stroke	4.7	7.0	0.65	(0.10-4.11)	0.21*	0.615
Head trauma	11.6	7.0	1.75	(0.39-7.85)	0.55*	0.458
Primary neoplasm of CNS	0.0	0.0	-		-	-
Metastasis to CNS	0.0	0.0	-		-	-
Migraine	2.3	0.0	-		1.01*	0.314
Multiple sclerosis	0.0	0.0	-		-	-
Epilepsy	9.3	2.3	4.30	(0.46-40.23)	1.91*	0.167
Parkinson's disease	4.7	2.3	2.09	(0.18-23.47)	0.34*	0.557

χ^2 with Yates' correction; COPD: Chronic obstructive pulmonary disease; SLE: Systemic lupus erythematosus; TB: tuberculosis; HIV: Human immunodeficiency virus; AIDS: Acquired immunodeficiency syndrome; CNS: Central nervous system.

Table 2. Description of neuropsychological and neurological evaluation

	% AD Cases (n=43)	% Controls (n=43)	OR (CI95% OR)	χ^2	*p*-value
Neuropsychological evaluation	55.5	4.7	23.57 (5.05-110.03)	24.87*	<0.001
Neurological evaluation	46.5	4.7	17.82 (3.82-83.20)	19.79*	<0.001
Physical examination signs					
Palmar grasp reflex	60.5	2.41	62.71 (7.89-499.83)	33.07*	<0.001
Sucking reflex	55.8	0.0	-	33.05*	<0.001
Glabellar reflex	44.2	14.3	4.75 (1.66-13.62)	9.8*	0.007
Palmomental reflex	41.9	0.0	-	22.87*	<0.001
Gait abnormality	53.5	21.4	4.22 (1.63-10.90)	9.88*	0.007
Posture abnormality	51.2	19.0	4.45 (1.68-11.80)	10.19	0.006
Balance abnormality	42.2	14.3	4.75 (1.66-13.62)	9.80	0.07
Motor impersistence	41.9	0.0	-	22.87*	<0.001
Motor Perseverance	18.0	0.0	-	9.46*	0.009
Verbal Perseverance	27.9	2.4	15.87 (1.96-128.66)	11.34	0.003
Lower-limbs hypertonicity	20.0	0.0	-	26.05*	<0.001

Table 3. DSM-IVR Diagnostic Criteria for Alzheimer's disease

Diagnostic criteria	% Cases (n=43)	% Controls (n=43)	OR (CI95% OR)	χ^2	*p*-value
1.1 Memory Impairment	100.0	4.7	-	78.35*	<0.001
1.2 One or more of the following cognitive disturbances: aphasia, apraxia, agnosia, disturbance in executive functioning.	100.0	2.3	-	82.09*	<0.001
2. The cognitive deficits in criteria 1.1 and 1.2 each cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of social functioning.	100.0	0.0	-	86.0	<0.001
3. The course is characterized by gradual onset and continuing cognitive decline.	100.0	2.3	-	82.09	<0.001
4. The cognitive deficits in criteria 1.1 and 1.2 are not due to any nervous, systemic or substance-induced conditions.	100.0	0.0	-	86.0	<0.001
5. The deficits do not occur exclusively during the course of a delirium.	100.0	0.0	-	86.0	<0.001

Table 4. Body mass index in both groups

Category	% Cases (n=43)	% Controls (n=43)	OR (IC95% OR)	χ^2	*p*-value
Underweight	15.6	3.1	0.18 (0.01-1.88)	3.01*	0.389
Normal	62.5	68.8	1.00		
Overweight	9.5	12.5	1.21 (0.19-7.989)		
Obesity	12.5	15.6	1.14 (0.22-6.02)		

distribution was comparable to that found in the analysis of the total population (38,39).

Discussion

Many studies have been undertaken to identify potential risk factors for AD. Vascular factors such as arterial hypertension, dyslipidemia, hyperinsulinemia and DM2, obesity, subclinical atherosclerosis and arrhythmias have been associated with high risks of cognitive impairment and dementia (52). Studies in Latin America have also shown that metabolic syndrome doubles the risk of cognitive impairment (57). Kalaria (58) has indicated that the gradual adoption of westernized

Table 5. Description of variables measured quantitatively in both groups

Variables	Average (SD) Cases (n=43)		Average (SD) Controls (n=43)		t-test	*p*-value
Systolic Arterial Pressure (mm Hg)	129.9	(16.1)	128.5	(16.3)	0.08	0.771
Diastolic Arterial Pressure (mm Hg)	70.9	(11.0)	75.3	(10.1)	0.37	0.541
BMI	23.8	(7.0)	24.7	(4.2)	-068	0.493
Abdominal circumference (cm)	91.7	(12.2)	92.8	(12.3)	0.08	0.769
Glycemia (mg/dL)	90.8	(31.0)	80.7	(12.5)	3.31	0.072
Total cholesterol (TC) (mg/dL)	201.0	(36.9)	212.7	(37.5)	0.00	0.998
Triglycerides (TG) (mg/dL)	155.0	(64.5)	154.8	(67.7)	1.04	0.310
HDL (mg/dL)	47.3	(12.2)	57.1	(12.3)	0.07	0.931
LDL (mg/dL)	121.9	(31.1)	124.5	(35.4)	1.04	0.309
VLDL (mg/dL)	31.0	(12.9)	30.9	(13.4)	0.99	0.322
TC/HDL	4.3	(1.0)	3.9	(1.2)	1.35	0.248

mm Hg: millimeters of mercury; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; TC/HDL: total cholesterol/high-density lipoprotein ratio.

lifestyle involving excessive caloric intake, unhealthy diet, and decreased physical activity, favors the appearance of conditions such as AD. In the Honolulu-Asia Aging study (25), a significant association was found between diabetes, alone or combined with the *APOE* ε4, with an increase in the incidence of dementia and neuropathological outcomes in a cohort of 2,574 Americans of Japanese origin. This study associated diabetes with total dementia (RR 1.5, 95% CI 1.0-2.2) and with AD (RR 1.8, CI 1.1-2.9). Another study (59,60) found an RR of 1.3 (95% CI 0.9-2.1) for AD. Despite the large differences in the sizes of the sample populations, similarities occur between these reports and the current findings (the strength of a positive association between DM and AD) in which paired analysis found statistically significant correlation between AD and DM (χ^2 McNemar: 8.18, p= 0.004, with a minimum of 95% CI and OR of 1.90), suggesting that even at a small scale there is a significant correlation. In the most recently published study (24), Ohara *et al.* showed that subjects with diabetes display a higher incidence of all-cause dementia, AD, and vascular dementia than control subjects with normal glucose tolerance (all-cause dementia: adjusted hazard ratio [HR] = 1.74, 95% CI 1.19-2.53, *p*=0.004; AD [HR] = 2.05, 95% CI 1.18-3.57, *p*=0.001).

In the current study, the most common non-neurological comorbidities were as follows: arterial hypertension (53.5% for AD patients and 65.1% for control subjects), acute myocardial infarction (11.6% for AD cases and 4.7% for control subjects), chronic obstructive pulmonary disease (18.6% and 4.0%), hypothyroidism (18.6% and 11.6%), smoking (65.1% and 27.9%), alcohol consumption (39.5% and 16.3%), cancer (16.3% and 7.0%), heart failure (11.6% and 7.0%) and dyslipidemia (9.3% and 16.2%).

Type 1 and type 2 DM have been associated with a progressive decline in cognitive functioning, mainly in elderly patients (49,50,61). Toxic effects of high glucose levels are mediated through an enhanced flux of glucose through the polyol and hexosamine pathways, disturbances of intracellular second messenger pathways, an imbalance in the generation and scavenging of reactive oxygen species, and by advanced glycation of important functional and structural proteins (49). In addition, DM2 is a risk factor for atherosclerosis of the carotid and intracranial arteries, thus increasing the risk of stroke, cognitive decline, and dementia (62,63). In the current study, eleven AD cases (25.6%) and one control subject (2.3%) were given a diagnosis of diabetes--all DM2, representing a statistically-significant difference (*p*=0.002). However, these results do not imply any causal relationship. The observations are a "one-time-point" observation, and not a follow-up study, and therefore, one cannot conclude from these data that DM leads to an increase in the risk of developing AD. However, this conclusion was reached by Ohara *et al.* (24) in a 15-year follow-up study.

In the current study sample, smoking with an OR of 4.82 (95% CI 1.93-12.04) suggested it as a risk factor for AD. In general, the literature is inconsistent on this subject. For instance, several studies (64-66) have reported evidence linking smoking more

as a protective factor than as a risk factor for AD. The meta-analysis of 19 prospective studies by Anstey, *et al.* (67), a total of 27,374 participants evaluated for dementia, reported a relative risk of 1.79 (95% CI 1.43-2.23) for AD among smokers and nonsmokers (67). A number of studies reflect the lack of association between these factors (68,69). Given the increasing prevalence of dementia and the high burden of comorbidity, morbidity, and disability, an urgent need is developing to clearly identify whether smoking is a modifiable risk factor for AD.

Among the most prevalent chronic diseases is chronic obstructive pulmonary disease (COPD). In the current study, COPD was found in 18.6% of patients with AD compared with 7% in the control patients. However, the number of patients was not sufficient for concluding an association with COPD. Regarding the presence of cancer, no correlation appears between the presence of cancer and AD (16.3% *vs*. 7.0% control patients). These data are similar to those in other studies, e.g.,12.15% for AD patients versus 10.11% for control subjects (70).

Finally, the present study is to be considered a preliminary work in which a correlation between *APOE* genotype, DM, and AD was sought in a specific population. Studies are planned to extend these comparisons to a larger and more diverse population in future.

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Conflicts of interest

The authors declare not having any conflict of interest in the research reported here.

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