




# Relationship Between Genetic Variants of ACAT1 and APOE with the Susceptibility to Dementia (SADEM Study)

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## Abstract

One of the hypotheses that have emerged to explain the origin of dementia relates the disease with altered lipid metabolism, particularly cholesterol. To maintain cholesterol homeostasis, the ACAT1 enzyme has an important function to regulate the production of A $\beta$ . Moreover, APOE is the main cholesterol carrier in the brain, and it has been reported as a risk factor for this disease. This study evaluates the relationship between ACAT1 and APOE genetic variants with susceptibility for the development of Alzheimer's disease and other dementias. We examined four ACAT1 polymorphisms (rs2247071, rs2862616, rs3753526, rs1044925) and two in the APOE gene (rs7412, rs429358) in a group of 204 controls and 196 cases of dementia. Our results show one protective haplotype: CGCA (OR = 0.34, 95% CI = 0.23–0.46;  $p < 0.001$ ) and one risk haplotype: CGGA (OR = 1.87, 95% CI = 1.34–2.60;  $p < 0.001$ ) for the development of dementia. Subjects identified as APOE- $\epsilon 4$  allele carriers had a higher risk of developing dementia compared with non-carriers, OR = 13.33 (95% CI = 3.14–56.31). The results support the hypothesis that the ACAT1 gene, together with the APOE gene, plays an important role in susceptibility to the development of dementia and shows genetic characteristics of the Mexican population that can be used to identify the population at risk.

**Keywords** ACAT1 · APOE · Cholesterol · Dementia · Polymorphism

## Introduction

Dementia is a major cause of disability and dependency among older adults worldwide, affecting memory, cognitive abilities, and behavior, interfering with one's ability to perform daily activities [1]. It is a multifactorial disease with modifiable lifestyle-related risk factors and non-modifiable genetic risk factors. Several hypotheses have been proposed to explain its origin and progress; one of them is related to cholesterol metabolism, which plays a fundamental role in the physiology and brain function [2].

Some studies have revealed that high cholesterol levels promote the formation of A $\beta$  [3, 4]. Cholesterol homeostasis is regulated by a dynamic balance of its absorption, de novo synthesis, esterification, catabolism, and release [5].

The enzyme ACAT1 has an important role in cholesterol homeostasis, preventing over-accumulation of free cholesterol in the cells, forming cholesteryl esters; it has been suggested that the balance between the esters and free cholesterol is a key point in the control of amyloidogenesis. ACAT activity has been associated with amyloid level and with the formation of brain amyloid plaques [6–14].

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APOE protein is an apolipoprotein whose main function is to participate in transport and delivery of lipids to target sites, so it also participates in cholesterol homeostasis, and it has been involved in the aggregation and elimination of A $\beta$  [15]; in addition,  $\epsilon 4$  allele of *APOE* gene has been identified as the strongest genetic risk factor for dementia [16–19].

Thus, given that APOE and ACAT1 have an important function in cholesterol homeostasis in the brain, modifications in its enzymatic activity generated by the different genetic variants could be related to the start or progress of dementia; however, no conclusive results have been obtained, because while some of the studies found an association [20, 21], others report negative results [22, 23].

The objective of the present study was to evaluate the relationship between *ACAT1* and *APOE* genetic variants with susceptibility for the development of Alzheimer's disease and other dementias, vascular dementia, and mixed, in the Mexican population.

## Material and Methods

### Study Population

Data of the present study were obtained from the Study of Aging and Dementia in Mexico (SADEM) [24, 25]. Our study included a total of 400 individuals, of whom 204 were considered as controls and chosen at random from among population older than 60 years with a negative diagnosis of any type of dementia or cognitive problems and 196 with dementia (109 cases of Alzheimer's disease, 59 vascular dementia, and 28 mixed).

The diagnosis of dementia was made in two steps. First, all participants of SADEM study were screened with the Mini Mental State Examination (MMSE) [26]; those participants with a cutoff  $\leq 24$  underwent a battery of neuropsychological tests and a standardized neurological examination. To quantify the severity of symptoms of dementia, the Clinical Dementia Rating, or CDR [27], was used.

In the second stage, the diagnosis was confirmed by consensus expert panel review including neuropsychologists, neurologists, and geriatric physicians. Diagnosis was based on the Diagnostic and Statistical Manual of Mental Disorders criteria for dementia (DMS-IV-R), the National Institute for Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [28], and/or the National Institute of Neurological Disorders and Stroke Association Internationale Pour la Recherche et Enseignement en Neurosciences (NINDS-AIREN).

Alzheimer's disease (AD) was diagnosed as probable; diagnosis of vascular dementia (VaD) was performed using a two-step procedure: (1) diagnosis of dementia and (2)

association of dementia to lesions of vascular origin. The criteria for a diagnosis of mixed dementia (AD/VaD) were that the course was suggestive of AD, and the patient had focal neurologic symptoms suggestive of ischemia. The presence of vascular risk factors alone in a patient with otherwise clinically typical AD would not be enough to support a diagnosis of AD/VaD [25]. A requirement for inclusion was that the participant be mestizo born in Mexico, and candidates were only included after knowing the characteristics of the study and agreeing to participate with a signed informed consent. The research protocol has been approved by The National Commission of Scientific Research, as well as by the IMSS Ethics Commission, registration number R-2015-785-012.

### Clinical Characteristics

All included older adults were evaluated through a specific interview for this study, which included the following data: sex, age, education, body mass index (BMI), which was calculated using height and weight, comorbidity evaluated using the Charlson comorbidity index [29], and alcohol and tobacco consumption. Systolic and diastolic blood pressures were measured using an automatic blood pressure monitor Microfile BP A100.

Body composition was assessed with a 353ioi JAWON Body Composition Analyzer. Bioelectrical impedance was analyzed in the morning after a 12-h fast and adequate hydration, with the patient standing up and wearing light clothing, without shoes. The analyzer measured weight with an accuracy of within 0.1 kg, as well as body impedance (in ohms), with calculation of percentage of total body fat and visceral fat area in cm<sup>2</sup>.

### Biochemical Analysis

Venous blood samples were obtained from all subjects after a fast equal to or greater than 10 h and processed at the clinical chemistry laboratory of the National Medical Center, IMSS. The levels of glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-c), and triglyceride levels were determined in serum through the semiautomatic chemical analyzer Ekem Control Lab. The low-density lipoprotein cholesterol (LDL-c) was calculated with Friedewald's formula modified by De Long [30].

### DNA Amplification and Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the method proposed by Lahiri and Numberger [31]. DNA samples were stored at  $-70$  °C until analysis. To verify the concentration and purity of the DNA, the NanoDrop® 1000 spectrophotometer was used at a wavelength of 260 and 280 nm. To select the SNPs for this study,

we considered those previously reported in the literature website ([www.ncbi.nlm.nih.gov/SNP](http://www.ncbi.nlm.nih.gov/SNP)), choosing those that had an LD threshold of  $r^2 \leq 0.1$ , a 1 Mb interval, and a minor allele frequency (MAF)  $\geq 1\%$ . Four SNPs, rs2247071, rs2862616, rs3753526, and rs1044925, for *ACAT1* and rs429358 and rs7412 for *APOE*, were selected (<http://www.appliedbiosystems.com/>). In addition, the polymorphisms of the two rs429358 and rs7412 chosen result in three major isoforms  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  of APOE.

To get information about each polymorphism, see Table 1. The samples were genotyped using 5-exonuclease TaqMan genotyping assays on ViiA 7 Real time PCR System, according to manufacturer's instructions (Applied Biosystems, CA, USA).

*APOE* was typed using real-time PCR by Applied Biosystems (Foster City, CA). Detection was performed based on the fluorescence intensities of FAM and VIC. We use standard DNA controls on each genotyping plate in both the real-time PCR assays.

## Statistical Analysis

Quantitative variables are expressed as mean  $\pm$  standard deviation, medians, and interquartile ranges. Qualitative variables are expressed as percentages. Allelic and genotypic frequencies were obtained by direct counting, and the Hardy-Weinberg equilibrium (HWE) of SNPs in cases and controls was calculated with Pearson's Chi-square test and Fisher's exact test. We use non-parametric Kruskal-Wallis test for comparing three genotype groups and the non-parametric Mann-Whitney test for comparing two genotype groups.

According to the number of copies of an allele necessary to modify the risk conferred, five inheritance models were considered: codominant, dominant, recessive, over-dominant, and log-additive. This was selected as the best model to show the lowest value corresponding to the Akaike information criterion (AIC). With the selected inheritance model, multiple logistic models were constructed integrating the variables with biological relevance or statistical significance, as well as the potentially confusing variables (age, sex, fatty visceral, and glucose). To test genotyping association between *ACAT1*

SNPs and dementia, odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using logistic regression. The estimation of haplotypes, pairwise linkage disequilibrium parameter ( $D'$ ), and haplotype blocks were performed with the HaploView program ([www.broad.mit.edu/mpg/haploview](http://www.broad.mit.edu/mpg/haploview)). The level of statistical significance was set at  $p < 0.05$ .

## Results

### Study Population

The clinical and demographic characteristics of study groups are shown in Table 2. The total percentage of women was 64.5%, without significant differences between both groups. In the control group, the median age was 70 (65–76) years, while in the group with dementia, it was 77 (71–83) years ( $p < 0.001$ ).

When comparing between-study groups, individuals with dementia showed a lower level of education ( $5.71 \pm 5.49$  vs.  $6.55 \pm 5.09$  years;  $p = 0.025$ ), elevation in glucose levels (112 (95.5–149) vs. 97 (90.5–105.5);  $p < 0.001$ ), and in general a decrease ( $p < 0.05$ ) in the levels of variables related to body composition such as BMI, body fat and visceral fat area.

In terms of habits, both smoking and alcohol consumption were more common in the control group (38.73 vs. 28.72;  $p = 0.035$  and 34.80 vs. 18.88;  $p < 0.001$ ; respectively).

There was no significant difference between the groups in the levels of total cholesterol, HDL-c, triglycerides, hypertension, and diabetes mellitus.

### Analysis of Polymorphisms

Genotype frequencies of the polymorphic studied for *ACAT1* are shown in Table 3. The G allele of rs3753526 and C allele of rs1044925 genotype were significantly higher in the dementia group ( $p < 0.05$ ). The presence of the minor allele was observed with greater frequency in the control group for polymorphisms rs3753526 and rs1044925. With rs3753526

**Table 1** Polymorphism information

No. <sup>a</sup>	Chr	Gene	dbSNP <sup>b</sup>	Allele change (ancestral allele)	Residue change amino acid [codon]	Chr. position (bp)	Location in gene
1	1	ACAT1	rs2247071	T>C	N/A	179300309	Intron variant
2	1	ACAT1	rs2862616	A>G	N/A	179326397	Intron variant
3	1	ACAT1	rs3753526	C>G	L[CTC] $\Rightarrow$ L[CTG]	179350406	Coding sequence variant
4	1	ACAT1	rs1044925	C>A/C>G	N/A	179354603	3 prime UTR variant
5	19	APOE	rs7412	C>T	R[CGC] $\Rightarrow$ C[TGC]	17667948	Coding sequence variant
6	19	APOE	rs429358	T>C	C [TGC] $\Rightarrow$ R [CGC]	44908684	Coding sequence variant

<sup>a</sup> Order of the polymorphisms is according to the chromosomal position in each gene

<sup>b</sup> SNP ID in database dbSNP

**Table 2** Clinical, demographic, and genetics characteristics

Variable	All ( <i>n</i> = 400)	Controls ( <i>n</i> = 204)	Dementia ( <i>n</i> = 196)	<i>p</i> value*
Gender, <i>n</i> (M%/W%)	142(35.50)/258(64.50)	74(36.27)/130(63.73)	68(34.69)/128(65.31)	0.741
Age, years	74 (67–80)	70 (65–76)	77 (71–83)	< 0.001
Education level, years	6.14 ± 5.30	6.55 ± 5.09	5.71 ± 5.49	0.025
BMI, kg/m <sup>2</sup>	26 (23.55–30)	26.70 (24.40–30.40)	25.60 (23.30–28.90)	0.013
Glucose, mg/dL	101 (93–126.50)	97 (90.50–105.50)	112 (95.50–149)	< 0.001
Total Cholesterol, mg/dL	203 (180–226)	202 (179.50–226)	204 (182.50–226)	0.822
HDL-c, mg/dL	51 (43–60.50)	51 (42–60)	52 (44.5–61)	0.193
LDL-c, mg/dL	117 (93–138)	120 (96–139)	112 (89–134)	0.039
Triglycerides, mg/dL	144 (115–192)	144 (114.50–192)	145 (117–191.50)	0.873
Body fat, %	32 (26.6–37.8)	33.9 (26.8–39.1)	31.3 (25.75–36.5)	0.024
Visceral fat area, cm <sup>2</sup>	138.5 (113–188)	148 (117–202.5)	133 (103–173.5)	0.002
Charlson index	3 (2–4)	2 (2–4)	3 (2–5)	0.002
Hypertension, <i>n</i> (%)	295 (73.75)	148 (72.55)	147 (75.00)	0.578
Type 2 diabetes mellitus, <i>n</i> (%)	146 (36.5)	70 (34.31)	76 (38.78)	0.354
CVA, <i>n</i> (%)	30 (7.50)	7 (3.43)	23 (11.73)	0.002
Smoking habit, <i>n</i> (%)	135(33.83)	79(38.73)	56(28.72)	0.035
Alcohol consumption, <i>n</i> (%)	108(27.00)	71 (34.8)	37(18.88)	< 0.001
APOE				
ε2ε2	23 (5.75)	4 (1.96)	19 (9.69)	0.007
ε3ε3	252 (63.0)	156(78.47)	96(48.98)	< 0.001
ε4ε4	18(4.5)	2(0.91)	18(9.18)	< 0.001
ε2ε3 or ε3ε2	90 (22.5)	42 (20.59)	48 (24.49)	0.402
ε2ε4 or ε4ε2	0	0	0	0
ε3ε4 or ε4ε3	17 (4.25)	2(0.98)	15(7.65)	< 0.001

Data are shown as mean ± standard deviation or median 25th and 75th percentiles, for qualitative variables the number of individuals and percentage, *n* (%)

*BMI* body mass index, *HDL-c* high-density lipoprotein cholesterol, *LDL-c* low-density lipoprotein cholesterol, *CVA* cerebrovascular accident

\**p* value was estimated using the Mann-Whitney *U* test and the Chi-square test for qualitative variables

polymorphism, we identified a log-additive model while for rs1044925, the best model was dominant model.

We observed associations between two polymorphisms of *ACAT1* with risk of dementia for the site rs3753526, with the log-additive model of inheritance (OR = 1.98, 95% CI = 1.40–2.79, *p* < 0.001). Similarly, the site rs1044925 showed dominant model of inheritance (OR = 2.31, 95% CI = 1.53–3.49; *p* < 0.001).

Because the presence of the *T* allele for rs2247071 and the *A* allele for rs2862616 were not observed, they were not considered in the analysis.

On the other hand, with respect to *APOE* genotypes, the observed frequency of ε2ε2 genotype in control subjects was lower than that in dementia cases (1.96% vs. 9.69%; *p* < 0.05). The distribution of ε2ε3 genotype was not significantly different in both groups (76.4% in controls vs. 48.9% in dementia, *p* = 0.098). The ε3ε4 genotype frequency in dementia cases was

significantly higher than that in control group (7.6% vs. 0.9%, *p* = 0.001). The distribution of ε2ε4 genotype was similar in both groups. One different distribution of ε4ε4 genotype was observed between the groups, being significantly higher in dementia cases compared with controls (9% vs. 0, *p* = 0.034).

The subjects identified as *APOE*-ε4 allele carriers had a higher risk of developing dementia compared with non-carriers, OR = 13.33 (95% CI = 3.14–56.31); this risk was maintained after adjusting for sex.

Stratifying the data by the ε4 allele of *APOE* did not change the results of the *ACAT1* genotypes and alleles of dementia and control subjects (Table 4).

Based on the calculated effect sizes and with a significance level of 0.05 to detect differences in genotype frequencies between groups, our study sample had for Rs3753526 (allele 14%, genotype > frequency 69%) and Rs1044925 (allele 71%, genotype > frequency 97).

**Table 3** Association of ACAT polymorphisms with dementia

Polymorphisms	Allele frequency (%)		Genotype frequency (%)			Model	OR (95% CI)	p value	AIC
	C	G	CC	CG	GG				
<b>rs3753526</b>									
Dementia (n = 196)	274 (69.9)	118 (30.1)	99 (50.5)	76 (38.8)	21 (10.7)	Co-dominant	1.00	---	525.44
Controls (n = 204)	333 (81.6)	75 (18.4)	129 (63.2)	75 (36.8)	0 (0)	--	1.32 (0.87–1.99)	0.187	551.74
						Dominant	1.69 (1.13–2.51)	0.010	558.18
						Recessive	---	---	542.64
						Over-dominant	1.09 (0.73–1.63)	0.678	
						Log-additive	1.98 (1.40–2.79)	<0.001	
<b>rs1044925</b>	<b>A</b>	<b>C</b>	<b>AA</b>	<b>AC</b>	<b>CC</b>				
Dementia (n = 196)	277 (70.7)	115 (29.3)	101 (51.5)	75 (38.3)	20 (10.2)	Co-dominant	2.45 (1.56–3.84)	1.559	543.72
						--	1.91 (0.94–3.92)	0.935	542.11
Controls (n = 204)	334 (81.9)	74 (18.1)	145 (71.1)	44 (21.6)	15 (7.3)	Dominant	2.31 (1.53–3.49)	<0.001	557.34
						Recessive	1.43 (0.71–2.88)	0.315	544.92
						Over-dominant	2.25 (1.45–3.50)	<0.001	546.39
						Log-additive	1.72 (1.26–2.35)	0.001	

OR odds ratio, 95% CI 95% confidential interval, AIC Akaike information criterion

## Analysis of Haplotypes

The linkage disequilibrium plot for the four polymorphisms of *ACAT1* is shown in Fig. 1. We observed an association between the polymorphisms of rs3753526 and rs1044925 (OR = 1.95, 95% CI = 1.35–2.82 and OR = 2.12 95% CI = 1.37–3.29). We built haplotype block with rs2247071, rs2862616, rs2862616, and rs3753526, as shown in Table 5. The H1: CGCA haplotype showed a higher frequency in the control group, compared with the dementia group, which suggests a lower risk of developing the disease (OR = 0.34, 95% CI = 0.23–0.46;  $p < 0.001$ ), while the H2: CGGA haplotype is found in a higher proportion in the dementia group, suggesting a risk association with the disease (OR = 1.87, 95% CI = 1.34–2.60;  $p < 0.001$ ). In this case, the haplotypes H4: CACC, H5: TACC, and H6: CACA have a frequency in the population of less than 20%.

## Discussion

In this study, we analyzed the relationship of four polymorphisms of the *ACAT1* gene and the *APOE* gene with genetic susceptibility for the development of dementia. We found a

frequency of 0.04 for the *T* allele of the rs2247071 site and 0.10 for the *A* allele of the rs2862616 polymorphic site, low frequencies compared with those reported for the American population in the 1000 genomes project ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)), which are 0.13 for the *T* allele and 0.30 for the *A* allele. However, our results are based on an adequate power for our sample (with an alpha of 0.05, considering alleles, genotypes, and haplotypes) that was as low as 66% and as high as 99%. Also in the control group, these alleles are not present, probably due to the low frequency of the polymorphism observed in our population. The frequency of polymorphisms differs between populations, so it is important to carry out studies in the Mexican population.

Although they were found in a higher proportion in the cases and could be associated with the presence of the disease, the low frequency of the control group did not allow the corresponding mathematical calculations to be made.

In the study by Lamsa et al. [21], in Finnish population, the CC genotype of the rs2247071 site was in a higher proportion in the cases, increasing the risk of AD (OR = 1.4, 95% CI = 1.04–1.78;  $p = 0.029$ ) compared with the other genotypes. In our study, the *T* allele (CT and TT genotype) is the one found more frequently in the group of patients with dementia. These two polymorphic sites, rs2247071 and rs2862616, are found

**Table 4** Association of *ACAT1* polymorphisms with *APOE-ε4* carriers

Polymorphism	Dementia OR (95% CI) <sup>a</sup>	<i>APOE-ε4</i> carriers OR (95% CI)	<i>APOE-ε4</i> non-carriers OR (95% CI)
rs3753536	C 1.91 (1.37–2.66)	2.05 (1.5–8.30)	1.75 (1.24–2.48)
	G		
rs1044925	A 1.87 (1.34–2.61)	2.14(1.55–8.20)	1.87(1.32–2.65)
	C		

OR odds ratio, 95% CI 95% confidence interval

<sup>a</sup> Crude

**Table 5** ACAT1 haplotype frequencies in dementia and controls

Haplotype	Sequence <sup>a</sup>	Frequency		OR (95% CI)	<i>p</i> value
		Dementia <i>n</i> = 196	Controls <i>n</i> = 204		
H1	CG CA	0.375	0.635	0.34 (0.23–0.46)	< 0.001
H2	CG GA	0.295	0.184	1.87 (1.34–2.60)	< 0.001
H3	CG CC	0.134	0.181	0.71 (0.48–1.03)	0.074

OR odds ratio, 95% CI 95% confidence interval

<sup>a</sup> Order of the polymorphisms is according to the chromosomal position (rs2247071, rs2862616, rs3753526, rs1044925)

in an intronic region, the polymorphisms present in this region can have an impact on the structure and function of RNA, affecting the cutting and splicing process.

The rs3753526 polymorphic site is found in an exon; in this case, the base change does not cause an amino acid change, which is why it is called a synonymous mutation, but the polymorphisms present in these regions can also affect the cutting and splicing process and the effectiveness of this process. For rs3753526, the inheritance model that best fits is the log-additive, which indicates that having the *G* allele doubles the risk (OR = 1.98, 95% CI = 1.40–2.79) compared with the over-dominant. In the aforementioned study, in Finnish population, no association was found, and the frequency of the *G* allele was less than in our population (cases = 0.12 and controls = 0.11 vs. cases = 0.30 and controls = 0.18); in that study,

they did not report a relationship significant with Aβ42, tau, and ptau levels.

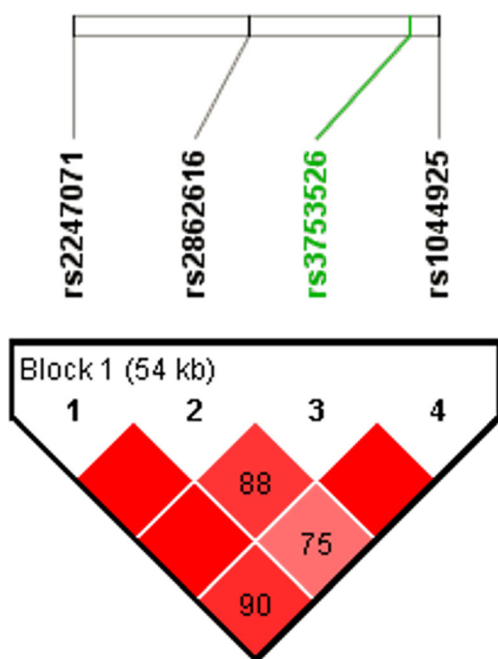
The rs1044925 polymorphism is located in the 3'UTR region, and polymorphisms in this region could affect the translation and stability of mRNAs [32]. *C* allele is over-represented in the dementia group. In studies carried out in Chinese and Finnish population [21, 23], it was reported that no association was found between polymorphisms and the disease. Our results agree with those reported by Wollmer [20], carried out in 309 patients with AD; they reported that *A* allele and the homozygous genotype for this allele were associated with a lower risk for AD (OR = 0.6, 95% CI = 0.4–0.8). That study also reported that *AA* genotype was associated with a low amyloid load ( $p = 0.030$ ) and a lower level of cholesterol in cerebrospinal fluid ( $0.46 \pm 0.17$  vs.  $0.76 \pm 0.26$  mg/dL;  $p = 0.005$ ). Wollmer et al. suggest that the *AA* genotype may be associated with reduced ACAT1 activity, and therefore, this would result in decreased production of cholesterol esters, causing decreased Aβ production [20].

To elucidate the association between the ACTA1 of the rs1044925 and Apo E4 distribution with AD by comparing AC + CC VS AA, we conducted a meta-analysis in which  $I^2$  of 0 was obtained, which reinforces the association identified in our study.

In the haplotype analysis, we identified a protection haplotype: CGCA and another risk: CGGA. The *G* allele of the rs3753526 site appears to be responsible for the risk modification between both. On the other hand, in the risks calculated for the alleles and genotypes, an associated risk of the *G* allele was identified, with a log-additive inheritance model with an OR = 1.98 (95% CI = 1.40–2.79), and the genotype homozygous *GG* only appeared in cases.

In a previous study [21], it was reported that rs2247071 and rs2862616 were as associated strongly with a  $D'$  value of 0.95; in our study, we also found a strong association, with  $D' = 1$  in both cases.

The frequencies that we got for the *APOE* alleles are similar to those previously reported, *APOE*-ε3 allele being the most frequent [33, 34]. In our study, the subjects identified as *APOE*-ε4 allele carriers had a higher risk of developing dementia compared with non-carriers, which is also in



**Fig. 1** Linkage disequilibrium map. Haploview linkage disequilibrium graph of ACAT1 gene. Pairwise linkage disequilibrium coefficients  $D' \times 100$  are shown in each cell ( $D'$  values = 1.0 are not shown). Standard color scheme of Haploview was applied for linkage disequilibrium color display ( $D' = 1$ , shown in bright red;  $D' < 1$  shown in shades of pink/red)

accordance with what has already been published and for what has been established as the main genetic risk factor for dementia [16–19].

The calculated risk for *ACAT1* genotypes remains unchanged between carriers and non-carriers of the *APOE-ε4* allele, although independently, the allele does represent a risk for dementia. This is in concordance with what has been reported, where the calculated risk for *ACAT1* polymorphism was independent of *APOE-ε4* status [20–23].

Finally, although our results showed a higher percentage of body fat, visceral fat area and BMI between the control compared with dementia group. Controversies persist about the associations of BMI with risk of dementia, although apparently, the risk is different depending on the age of the patients [33]. Overall weight changes are common in people with dementia; particularly, weight loss is common among dementia patients and this increases the risk of cognitive impairment and poorer disease outcomes [34, 35]. This observed decrease in BMI, body fat percentage, and visceral fat area in patients with dementia may be due to various factors as a decline in food intake, digestive problems, or swallowing difficulties and even the medication they receive to treat the concomitant diseases that accompany the dementia [35–37].

The results of this study allowed identifying risk variants, which represents the first step to establishing genetic markers; the clinically observed genetic associations can identify risk genes and candidate variants, but the causal relationships frequently remain unknown. Clarifying the molecular details is a fundamental step in optimizing clinical utility.

It is important to consider that in addition to the genetic factor, the environment and other factors have a fundamental role in the appearance of these diseases, and therefore, in those individuals with genetic susceptibility, lifestyle is decisive for the appearance of clinical manifestations; so, these factors must be analyzed together.

## Conclusions

This is the first association study linking the *ACAT1* gene and the *APOE* gene in the Mexican population. The *G* allele of the rs3753526 polymorphism and the *C* allele of rs1044925 were identified as risk variants for dementia and this remains unchanged between carriers of the *APOE-ε4* allele and *ε4* non-carriers. We also identified one risk haplotype and one protector haplotype for dementia.

The results indicate that *ACAT1* and *APOE* polymorphisms modify the risk of presenting dementia; this supports the hypothesis that altered cholesterol metabolism is involved in the development of pathology and may play an important role in the susceptibility of dementia for Mexican population. This information could expand the current knowledge of the genetic mechanisms involved in the development of dementia.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors that they have no conflict of interest.

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