Association between polymorphism c.1-765G>C of the COX2 gene and cognitive impairment in individuals 65 years or more with diabetes from a Geriatric Service in Monterrey, Mexico

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A B S T R A C T

Background and objective: Cognitive impairment and dementia are common geriatric syndromes in diabetic patients. Inflammation plays a crucial role in the pathophysiology of Alzheimer’s disease and cognitive impairment. Cyclooxygenases (COX) 1 and 2 participate in inflammation. The polymorphism c.1-765G>C of the COX2 gene might be protective against cognitive decline in Mexicans with diabetes mellitus through its reduced promoter activity. To determine the association between polymorphism c.1-765G>C of the COX2 gene and cognitive impairment in elderly adults with diabetes.

Patients and methods: Case-control study. We included diabetic patients from the Geriatric Clinic of General Hospital No. 17 who were over 65 years and accepted to participate. Cases were patients with a score of 24 or less on the Mini Mental Status Examination (MMSE) and with DSM IV criteria for dementia. Controls were those with MMSE scores of 25 or greater.

Results We included 97 patients (50 cases and 47 controls). There were no differences regarding clinical and laboratory characteristics between cases and controls. The frequency of the C allele and the GG genotype was higher in controls than in cases and this difference remained significant in a multivariate analysis with an odds ratio of 0.012 (95% CI 0.001–0.091) and 0.009 (95% CI 0.001–0.076) in the bivariate and multivariate analysis, respectively, using the GG genotype frequency as a reference.

Conclusion: Cognitive impairment in Mexican patients with diabetes is associated with less exposure to the GG genotype of the c.1-765G>C polymorphism of COX2.

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Asociación entre el polimorfismo c.1-765G>C del gen de la COX-2 y el deterioro cognitivo en individuos diabéticos de 65 o más años de un Servicio de Geriatría en Monterrey, México

R E S U M E N

Fundamento y objetivo: El deterioro cognitivo y la demencia son síndromes geriátricos frecuentes en los pacientes con diabetes. La inflamación es crucial en la fisiopatología de la enfermedad de Alzheimer y del deterioro cognitivo. Las ciclooxigenasas (COX) 1 y 2 participan en la inflamación. El polimorfismo c.1-765G>C del gen de la COX-2 protegería contra el deterioro cognitivo en adultos mayores diabéticos mexicanos por su menor actividad promotor. El objetivo de este estudio fue determinar la asociación entre el polimorfismo c.1-765G>C del gen de la COX-2 y el deterioro cognitivo en adultos mayores diabéticos.

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Introduction

Increased life expectancy has important implications for health systems worldwide. The projections indicate that between 1980 and 2050, life expectancy for people over 60 will increase 77%. Because of this, age-related diseases will increase. Among these, cognitive impairment with and without dementia represent conditions that directly affect the quality of life of the elderly population and may lead to increased use of health services. Cognitive impairment without dementia is a high-risk condition for the onset of dementia. The probability of developing the disease in an individual is 10–15% annually, in comparison with healthy controls, where dementia is 1–2% annually. The most serious complication of mild cognitive impairment is dementia with frequencies ranging around 7%. The most common presentation is Alzheimer's disease, which is considered a neurodegenerative disorder characterized in early stages by memory loss, and pathologically by deposits of amyloid plaques and neurofibrillary tangles.

There are multiple risk factors for cognitive impairment and dementia in Mexican population and worldwide, such as age, low education level, and genetic factors such as APOE; in this gene, single nucleotide polymorphisms (SNPs) at positions 112 (rs429358 and 158 (rs7412) determine three isoforms named APOE 2,3 and 4. APOE 4 is associated to risk, whereas APOE 3 is associated with protection. This association is important because in those who are homozygous, 55% at age 80 will have the disease, while only 27% of heterozygotes develop it; in addition, there is three-fold greater risk of developing cognitive impairment and Alzheimer's disease regardless of educational level, age and gender, although data have been described in Mexican population where unfortunately an association was not found. In addition to the above, the history of cerebrovascular disease and diabetes mellitus are noteworthy, since they represent a worldwide public health problem with a high prevalence in our country in the general population, and in elderly adults. Cognitive impairment and dementia in elderly adults with diabetes represent a catastrophe for the patient and family, since it is associated with an increased risk of functional dependence and complications. Diabetes mellitus and insulin resistance are linked to generalized inflammation and play a crucial role in the pathophysiology of Alzheimer's disease and cognitive impairment. Studies have shown an elevation of prostaglandins, inflammatory cytokines, and acute phase reactant proteins with increased activation of complement. This correlates with the presence of microglia and astrocytes in and surrounding amyloid protein plaques, a common finding in these patients. Cyclooxygenases (COX) are the main producers of prostaglandins from arachidonic acid. There are 2 types of COX enzymes. COX type 1 is involved in functions related to prostaglandin and thromboxane synthesis in the gastric mucosa. COX 2 is expressed mainly in the central nervous system and in inflammatory cells. In addition to the aforementioned, there are elevated levels of COX-2 in neurons of the hippocampal region and these are positively correlated with the density of the amyloid plaque and neurofibrillar tangles in this region. From a genetic standpoint, the COX2 gene has been mapped to 1q25, which is located between 1q23 and 1q31, two regions that have been genetically linked to Alzheimer disease. A functional polymorphism in the promoter region of the COX2 gene has been identified, a G to C transversion at position-765, which results in reduced promoter activity. It was shown that this polymorphism may be protective for the development of Alzheimer disease, besides being associated with protection against cerebral vascular disease and myocardial infarction, which in turn are linked to cognitive impairment.

However, to date, its presence has not been demonstrated in the Mexican population, nor has it been shown in patients with diabetes, who are at a greater risk than the general population. In addition, the joint presence of APOE, specifically type 4, and the COX2 polymorphism has not been evaluated. To date, no studies have been conducted in the Mexican population; therefore there is no information, a fact that motivated the present study.

Patients and methods

We carried out a case-control, observational, retrospective and analytical study, which included diabetic patients over 65 years of age that came for geriatric assessment during July 2009 to February 2010 and who accepted to participate. We excluded those with a Mini Mental Status Examination (MMSE) score less than 10, a score greater than or equal to 5 on the 15-item Yesavage Geriatric Depression Scale uncontrolled hypothyroidism or hyperthyroidism, acute confusional state, those using cinnarizine, chronic liver disease Child B or C, epilepsy or use of antiepileptics, antipsychotics, and with a creatinine level greater than 1.5. Cases were participants with cognitive impairment (MMSE < 25) and with a diagnosis of dementia made using DSM IV criteria. Controls were those with a normal neuropsychological evaluation (MMSE ≥ 25).

Variables

The variables analyzed were age, gender, years of schooling, history of smoking, family history of dementia and history of hypertension. We also counted the number of drugs being taken, determined the Barthel functional index, the MMSE score, glucose, total cholesterol, HDL, LDL and triglyceride levels.

General study procedure

After obtaining authorization in writing from the appropriate ethics committee, patients from the geriatric clinic were invited to participate in the study. Those who agreed to participate
underwent a comprehensive geriatric assessment, which included the above variables coded in a data collection instrument. Also, after completion of the assessment, a sample of capillary blood, about 10 μl, was obtained from the little finger of the non-dominant hand; the sample was then stored at 4 °C prior to analysis. Also, laboratory test results were collected. All participants provided informed consent. The participation of each patient concluded at the time of blood sample collection.

Determination of genetic polymorphisms

Determination of the c.1-765G>C polymorphism of the COX2 gene

Genotyping was performed from capillary blood, which was subjected to alkaline lysis with NaOH, followed by amplification of the polymorphic fragment by conventional PCR endpoint analysis using GoTaq Master Mix reagent (Promega, Inc., Madison, WI). The reaction mixture had a volume of 15 μl with the following composition: GoTaq Master Mix, 7.5 μl; water, 5.7 μl; sense primer, 0.3 μl; antisense primer, 0.3 μl; and blood alkaline lysate, 1.2 μl. The primers used at a concentration 10 μM were GGCTGTA-TATCCTGCTATATGC (sense) and CGGCTTTCTTGTCATCATG (antisense).24 A BIO-RAD C-1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA) was used. The amplification program was: 55 cycles of denaturation at 95 °C for 45 s, annealing at 59 °C for 45 s, extension at 72 °C for 45 s. After amplification, the products were digested with the restriction enzyme AciI (New England BioLabs, Ipswich, MA) that cuts the wild G allele. In this way, the mutated homozygous CC is not digested and is observed as a single band of 306 base pairs, the homozygous wild GC is cut and observed as two bands of 118 and 188 bp, and the heterozygous CT results in three fragments of 118, 188 and 306 base pairs. Digestion products were analyzed by submarine electrophoresis in 2% agarose gel stained with ethidium bromide and visualized in an ultraviolet transilluminator and recorded by digital photography with an orange filter. Genotypes were determined by two independent observers.

Determination of the APOE gene polymorphism

The APOE genotype was determined in the same way as the c.1-765G>C polymorphism of COX2, except that the annealing temperature was 58 °C and digestion was performed with the HhaI enzyme; the restriction pattern of this enzyme allows differentiation of six possible genotypes (APOE 22, 23, 24, 33, 34 and 44). The primers used were those described by Hixon and Vernier,33 except that the antisense primer was four bases longer.34 They were: TAAGCTGACGCGTTCAGAGA (sense) and ACAGAATTGGCAGGCTCTGGTACACTGCC (antisense).

Statistical analysis

We used descriptive statistics with measures of central tendency and dispersion to characterize the participants. For quantitative variables means ± standard deviation were used and for qualitative variables we used absolute frequencies and percentages comparing these with Student’s t test and chi square, respectively. We measured the frequency of genetic variants in cases and controls. We measured the association of polymorphisms with odds ratios and 95% confidence intervals. A multivariate analysis of variables associated with cognitive impairment was performed. The sample was calculated using the statistical package STATA v11.0, with an alpha of 0.05, and a power of 0.8, to estimate a difference in proportions of 0.7–0.4 for a minimum of 49 participants per group.

Results

One hundred patients over 65 years with diabetes were seen at the Geriatric Department of General Hospital No. 17 of the Mexican Social Security Institute (IMSS) between July 2009 and February 2010. In this period, there were a total of 100 patients, which corresponded to 50 cases and 50 controls. Three participants were eliminated from the control group because the blood sample was insufficient.

With regard to the comparative analysis of the general characteristics (see Table 1), there was no significant difference in terms of clinical variables such as age, gender, education, history of smoking, and dementia, as well as the duration of diabetes and a history of hypertension.

On the other hand, when comparing the clinical and laboratory characteristics (see Table 2), no significant difference in terms of laboratory variables such as glycemia, total cholesterol,
LDL-cholesterol, HDL-cholesterol, triglycerides, and albumin was found. There was a significant difference between test scores of the MMSE and the Barthel functional index.

Finally, when comparing the genotype frequencies of COX2 and APOE (see Table 3), the CG genotype of COX-2 was present in 2% of cases and 68% of controls (P < 0.001), with an odds ratio of 0.012 (95% CI 0.001–0.091), with the GG genotype frequency as a reference. APOE genotype 33 was present in 100% of cases and in 87.2% of controls, APOE genotype 34 in 0% of cases and in 10.6% of controls, and APOE genotype 44 in 0% in cases and 2.1% in controls (P = 0.033). In multivariate analysis with logistic regression, it was found that only the CG genotype of COX2 was associated with cognitive impairment (see Table 4).

### Discussion

The aim of this study was to determine the association between the genotype frequency of c.1765G>C polymorphism and cognitive impairment in elderly adults with diabetes mellitus. When the clinical and laboratory characteristics were compared, no significant difference between cases and controls was found, except for the score on the MMSE and the Barthel Index (an expected result since cognitive impairment causes a low Barthel functional index) thus one variable influences the other; therefore we can consider that the population is comparable in terms of their general characteristics.

When comparing the genotype frequencies, the CG and the C alleles was more frequent in controls, this being consistent with the findings by Abdullah et al. in their study of 329 elderly adults without diabetes, with and without Alzheimer’s disease, the GC genotype, as well as the C allele, were more common in controls than in cases. This study, however, is not comparable with ours because the population in our study had diabetes, and were Mexicans with an increased risk of cognitive impairment in contrast with those in the study by Abdullah et al. In that study, a higher frequency of the CC genotype in controls than in cases was found, although less than the CG genotype, in contrast with the present study that did not find this genotype, most likely due to the limited sample size.

Regarding the hypotheses to explain the reason for the more frequent CG genotype in controls, inflammation plays an important role in the pathophysiology of cognitive impairment, since these individuals are likely to have lower levels of systemic inflammation (not evaluated in this study). Another possible hypothesis to explain the association found is that this polymorphism is located in proximity to genes that have previously shown an association with Alzheimer’s disease on chromosome 1, and a condition that may be due to an imbalance between this polymorphism and another associated gene.

With regard to the presence of APOE, genotype frequency 33 was more frequently found in cases than in controls and genotype frequencies 34 and 44 in controls; this is in contrast with the findings by Villalpando Berumen et al. in Mexico City, who did not find an association with Alzheimer’s disease. One explanation for this is that in the present study, we included only patients with diabetes mellitus and there was no matching between the characteristics of the participants. Also, these were not comparable because they were not from the community, as was the case with Villalpando Berumen et al.

Regarding the overall analysis of genotype frequencies for the COX2 and APOE polymorphism using logistic regression, only the C allele of COX2 remained significant. One possible explanation is that the lower frequency of the C allele of the COX2 gene plays a more important role and has more weight in the pathophysiology of cognitive impairment. It is important to point out that there are no reports to date worldwide of the simultaneous measurement of both genotype variables in conjunction with other confounding variables (as in our study).

The main limitations of this study are those related to case-control studies, which may have selection bias, and recall bias, due to the retrospective nature and because glycated hemoglobin was not included as a possible confounding variable. In addition, this study did not extensively evaluate cognitive function with a battery of neuropsychological tests, which are a standard item of an evaluation. However, these are only used in case of doubt, and not systematically in all patients, without mentioning the fact that they are expensive and require application by a trained neuropsychologist, something that the Mexican Social Security Institute lacks. Another limitation of this study is that prostaglandin levels

### Table 3

Association of the COX2 and APOE polymorphism with cognitive impairment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cognitive impairment n = 50</th>
<th>Without cognitive impairment n = 47</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>1 (2.0%)</td>
<td>30 (63.8%)</td>
<td>&lt;0.001</td>
<td>0.012 (0.001–0.091)</td>
</tr>
<tr>
<td>GG</td>
<td>49 (98.0%)</td>
<td>17 (36.2%)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>CC</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>50 (100%)</td>
<td>41 (87.2%)</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>0 (0%)</td>
<td>5 (10.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>0 (0%)</td>
<td>1 (2.1%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR: odds ratio; 95% CI: 95% confidence interval.

### Table 4

Logistic regression analysis of allelic and genotype frequencies for COX2 and APOE genotypes 33, 34 and 44 for cognitive impairment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG Genotype of COX 2</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>0.001</td>
</tr>
<tr>
<td>GG Genotype of COX 2</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>APOE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>1.000</td>
<td>6.09E+12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>34</td>
<td>1.000</td>
<td>2.371</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

OR: Odds ratio; 95% CI 95% confidence interval.
were not assessed, so we cannot draw inferences with regard to cognitive impairment and its association with prosta glandin levels. This study has many strong points, such as that it simultaneously evaluates multiple variables associated with cognitive impairment as well as genetic analyses blinded to clinical results, besides being the first report of its kind in patients with diabetes mellitus.

**Conflict of interest**

The authors declare that they have no conflicts of interest.

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