The Methylenetetrahydrofolate Reductase C677T (rs1801133) and Apolipoprotein A5-1131T>C (rs662799) Polymorphisms, and Anemia Are Independent Risk Factors for Ischemic Stroke

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Background: Although there is adequate knowledge as to the role of traditional cardiovascular risk factors on stroke incidence, knowledge of other risk factors, particularly genetic ones, is still incomplete. *Methods:* To assess the participation of some polymorphisms, along with other modifiable risk factors, a case–control study was conducted. A total of 253 cases were identified in the emergency room of a general regional hospital, with a clinical trait of stroke confirmed by a skull computerized axial tomography scan. In the surgery ward, 253 controls were identified, gender and age (±5 years) matched. Biochemical parameters were measured, and 4 polymorphisms were genotyped by polymerase chain reaction, rs1801133 (methylenetetrahydrofolate reductase [MTHFR]), rs1498373 (dimethylarginine dimethylaminohydrolase type 1 [DDAH1]), rs662799 (apolipoprotein A5 [APOA5]), and rs1799983 (endothelial nitric oxide). Odds ratios were estimated to assess the strength of association, with 95% confidence intervals, both in a matched case–control analysis and in a conditional regression analysis. *Results:* Cases had higher mean blood pressure and triglycerides and lower hemoglobin levels. Heterozygous and homozygous subjects to the rs1801133 variant of the MTHFR gene had a 3-fold higher risk of stroke. In the dominant model, those with the polymorphism rs662799 of the promoter region for APOA5 had twice the risk of stroke. Anemia increased the risk of stroke 4-fold. *Conclusions:* Polymorphisms of the genes MTHFR (rs1801133) and APOA5 (rs662799), as well as anemia, are independent risk factors for stroke in Mexicans, together with traditional cardiovascular risk factors such as high triglycerides and high blood pressure. **Key Words:** Stroke—polymorphism—genetic—apolipoproteins A—methylenetetrahydrofolate reductase—anemia—risk factors.

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Introduction

The prevalence of traditional risk factors explains the occurrence of ischemic stroke in different populations.[1,2] These are modifiable risk factors as they are closely related to lifestyle, and reduction in their occurrence aids in decreasing the incidence of strokes.[3]

Other risk factors, such as high serum levels of homocysteine, have been associated with the occurrence of cardiovascular disease.[4] Although serum levels of homocysteine are closely related to nutritional factors,[5] there are genetic alterations associated with high blood levels of this protein. Specifically, variants of the gene for methylenetetrahydrofolate reductase (MTHFR) reduce enzymatic activity by 70% and increase homocysteine levels. It has been reported that this variant is relatively frequent in Mexico.[6] Meta-analysis studies suggest that polymorphism is associated with high levels of homocysteine in the blood but does not increase the risk of cardiovascular disease.[7] Notwithstanding these findings, recent studies found an association of polymorphism with ischemic heart disease,[8] as well as with stroke,[9] pointing to a causal relationship with the increase in levels of homocysteine in the blood.

It has been suggested that 5-methyl-tetrahydrofolate (5-MTHF) is responsible for the increase in the risk of cardiovascular disease and stroke, more so than with the levels of homocysteine. 5-MTHF has several antiatherogenic effects and increases the bioavailability of endothelial nitric oxide in vessels.[10] This is why other polymorphisms related to the synthesis of nitric oxide or of endothelial nitric oxide have been studied in regard to strokes.[11,12] Polymorphisms related to high levels of triglycerides and stroke have also been identified.[13]

To date, the relationship of genetic markers to the occurrence of strokes has been inconsistent and differential, to a certain extent, in the populations that have been studied. Although there is adequate knowledge as to the occurrence of traditional and modifiable cardiovascular risk factors, knowledge of other risk factors, particularly genetic ones, is still incomplete.

Material and Methods

A case–control study was performed at a medical specialty hospital. The study was approved by the institutional review board of the hospital and an informed consent form was signed. The cases involved patients who had been admitted to the hospital's emergency room presenting with clinical signs of an ischemic, acute, stroke, characterized by clinical symptoms or signs of focal or global alterations of brain function. Neurological damage was confirmed through a simple computerized axial tomography (CAT) scan of the skull.[14] Controls were obtained from the hospital's general surgery department, paired by age (±5 years) and gender with the cases (hospital-based controls), considering that controls should represent the source population of the cases with respect to exposure, and no main diagnosis for the surgical procedure was related to stroke risk factors.[15]

A blood sample was taken from all patients, the morning after admittance in a fasting state of at least 8 hours, for biochemical analysis of hemoglobin, glucose, total cholesterol, and triglycerides. None of the studied subjects had received any blood transfusion. For DNA extraction from peripheral blood, a QIAamp (QIAGEN, Hilden, Germany) kit was used following the manufacturer's instructions. DNA concentration was measured using optical density (VICTOR 3 1420 spectrophotometer, Perkin-Elmer, MA) and its integrity through .8% agarose gel, stained with ethidium bromide, electrophoresis (Gel Doc 2000, Bio-Rad, CA). Blood pressure was measured and body mass index was estimated. Anemia was defined as a hemoglobin value less than 120 g/L in females and less than 130 g/L in males.

Polymorphisms rs1801133 of MTHFR, rs1498373 of enzyme dimethylarginine dimethylaminohydrolase type 1 (DDAH1), rs662799 of the promoter region of apolipoprotein A5 (APOA5), and rs1799983 of the synthetase enzyme for endothelial nitric oxide (eNOS) were genotyped with the polymerase chain reaction technique using the TaqMan SNP genotyping assay (Applied Biosystems, CA). The genotyping success rate was at least 98%. Thirty percent of the samples were included in duplicate and genotyping had 100% concordance.

Statistical analysis was done using the Student *t* test to compare the means. Through a matched case–control analysis, the odds ratio (OR) was estimated as the measure of the association, with 95% confidence intervals (CI95%). To assess the independent participation of each studied risk factors, we carried out a multivariate conditional logistical regression analysis, with the OR estimated with CI95%. Variables included in the multivariate model were those risk factors that showed a relation to stroke in the bivariate analysis.

Results

A total of 253 cases and 253 controls were studied. Subjects with a stroke had higher mean values for blood pressure and for blood triglycerides, and lower mean values for hemoglobin. There were no differences in glucose or cholesterol values, or in the body mass index (Table 1).

Table 2 shows the distribution of polymorphisms in cases and controls. The low prevalence of the homozygous to the variant for rs662799 (APOA5) and rs1799983 (eNOS) is noteworthy. Hardy–Weinberg equilibrium was evaluated with an exact test available at http://www.husdyr.kvl.dk/htm/kc/popgen/genetik/applets/kitest.htm (accessed March 2017).

Also, 60.87% of cases had a history of high blood pressure, whereas in the controls the percentage was 45.45%