Genetic Risk Factors in Cerebrovascular Disorders and Cognitive Deterioration

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Abstract: Introduction: The study of variations in genes involved in the different events that trigger the atherogenic process, such as lipid metabolism (modification of LDL-cholesterol), endothelial function and hypertension, immune response (recruitment of macrophages and cell formation) and stability of atherosclerotic plaques (thrombosis), established the risk for suffering a vascular disorder. A total of 2455 cases over 50 years of age were genotyped for a panel of 19 SNPs in 15 genes encoding for proteins involved in the atherogenic process. This study shows the relevance of polymorphisms in APOB (odds ratio (OR), 1.17; 95% confidence interval (95% CI), 0.74-1.85), APOC3 (OR, 1.33; 95% CI, 0.82-2.17) and APOE (OR, 1.75; 95% CI, 1.09-2.80), as genetic risk markers for hypercholesterolemia; polymorphisms in ACE (OR, 1.68; 95% CI, 0.32-8.77) and AGT (OR, 1.74; 95% CI, 0.97-3.14) for hypertension; and in APOE*3/*4 (OR, 2.06; 95% CI, 1.70-2.51) and APOE*4/*4 (OR, 3.08; 95% CI, 1.85-5.12) as unambiguous markers of dementia.

Result: Our results also showed the transversal importance of proinflammatory cytokines in different stages of atherogenesis, with special relevance of IL6 (OR, 1.39; 95% CI, 0.56-3.49) and TNF (OR, 1.40; 95% CI, 0.92-2.15) related to hypercholesterolemia and hypertension. The set of markers involved in this genetic risk panel makes it a powerful tool in the management of patients with different vascular disorders.

Keywords: Dementia, Genetic risk, Hypercholesterolemia, Hypertension, Inflammation, Thrombosis, Vascular risk.

1. INTRODUCTION

Cerebrovascular disorders are due to abnormalities that affect the blood flow and cause damage to brain tissue due to the total or partial absence of blood supply. Such diseases occur when the arteries carrying oxygenated blood to the brain become damaged or blocked due to plaque deposits. This plaque may completely block cerebral blood flow causing a stroke that causes complications of varying magnitude, from transient ischemic attacks to massive bleeding.

Different authors have considered atherosclerosis as a form of chronic inflammation resulting from the interaction between modified lipoproteins, macrophages, lymphocytes and other normal elements of the arterial wall [1, 2]. This inflammatory process can ultimately lead to the development of plaques that appear in the arterial lumen. Several epidemiological studies have made it possible to detect the main genetic and environmental risk factors related to stroke [3].

Genetic risk of cerebrovascular diseases is likely to be polygenic (Fig. 1) and multifactorial, with environmental factors playing a very important role.

High levels of low-density lipoprotein (LDL) are markedly related to the development of atherosclerosis. It is generally accepted that atherosclerotic lesions are initiated via the enhancement of LDL uptake by monocytes and macrophages [4, 5]. Allelic variants for apolipoproteins such as APOB, APOCIII and APOE, as well as the cholesterol ester transfer protein (CETP) and the lipoprotein lipase (LPL), play a key role in lipoprotein metabolism and are linked to the development of atherosclerosis and increased vascular risk [6-13].

Progression from the initial fatty streak to more complex lesions involves several changes in the artery wall. The genetic and environmental factors associated with the development of arterial hypertension are highly informative markers of the risk for developing cerebrovascular pathologies. Following are the enzymes that are related to the endothelial stability, such as the endothelial nitric oxide synthase (NOS3), which synthesizes nitric oxide from the amino acid arginine and is a constituent of vascular endothelial cells; the angiotensin-converting enzyme (ACE), which plays an important role in regulating blood pressure and electrolyte balance, and angiotensinogen (AGT), associated with an increased risk of essential hypertension, plays a crucial role in endothelial function and in profusion of the atherosclerotic plaque [14-18].
Circulating markers of inflammation are associated with the risk of atherosclerosis and stroke, although the reasons for these associations remain unclear. It is now widely recognized that atherosclerosis is a specific example of a chronic inflammatory response mainly to dyslipidemia and other risk factors. The foam cells and activated endothelium may also produce proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor alpha (TNF-α), which promote further development of the inflammatory response [19-25].

The last stage of the atherogenic process results in plaque rupture and thrombosis. At this stage, variations in coagulation factor II or prothrombin (F2), coagulation factor V Leiden (F5), and methylenetetrahydrofolate reductase (MTHFR), are especially important, increasing atherothrombotic risk [26-31].

The evolution of complex diseases, such as stroke, is based on the interaction of genetic and environmental factors. When we determine the genetic risk of a patient, we can classify it as a high-risk, treatable phenotype, and thus the physician can influence the modifiable environmental component in a more cost-effective way.

2. MATERIALS AND METHODS

2.1. Subjects

A total of 2459 patients over 50 years of age, who attended the outpatient clinic at the EuroEspes Biomedical Research Center from January 1995 to December 2015, was analyzed.

Patients were distributed according to three different parameters: hypercholesterolemia, hypertension and cognitive impairment. Hypertension was defined as having a systolic BP/diastolic BP equal to or greater than 140/90 mmHg. Hypercholesterolemia was determined by LDL-cholesterol/Total-cholesterol values equal to or greater than 160/240 mg/dL. Cognitive impairment status was assessed with the Mini-Mental State Examination (MMSE) and the inclusion criterion was an MMSE value below 25. Controls for hypercholesterolemia were patients with LDL-cholesterol/Total-cholesterol values less than 160/240 mg/dL; controls for hypertension were patients having a systolic BP/diastolic BP lesser than 140/90 mmHg; and controls for cognitive impairment were patients with MMSE values equal or higher than 25.

2.2. Genotype Analysis

DNA was extracted from peripheral blood using Qiagen extraction columns (Qiagen, Hilden, Germany). A total of 19 single nucleotide polymorphisms (SNPs) from 15 different genes (Table 1) was genotyped. RT-PCR amplification (Real-Time Polymerase Chain Reaction) was performed using TaqMan assays for single nucleotide polymorphisms (SNPs) using ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Waltham, Massachusetts, USA), StepOne Plus Real Time PCR System (Life Technologies, Waltham, Massachusetts, USA), and TaqMan® OpenArray® DNA microchips in QuantStudio™ 12K Flex Real-Time PCR System. OpenArray® genotyping analysis was performed using the Genotyper software (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.3. Statistical Analysis

The Pearson’s chi-square test was applied to test population sample deviation from the Hardy-Weinberg equilibrium (HWE). To test the association of SNPs with disease risk factors such as hypercholesterolemia, hypertension, and cognitive impairment, allelic and genotypic frequencies were analyzed using Pearson’s chi-square test and odds ratio (OR) calculation. Dominant, Recessive, and Multiplicative (allele counts) specific genetic models were tested to find the better explanation of the SNP association with disease.

2.4. Patient Consent

Written informed consent was obtained from all capable participants. In those considered to have reduced capacity to understand the informed consent document due to their cognitive deficits, a legal representative or caregiver consented on their behalf. This study and the consent procedures were approved by the institutional review board of the EuroEspes Medical Center, in line with the ethical code of the World Medical Association (Declaration of Helsinki) and the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals of the International Committee of Medical Journal Editors (ICMJE).

3. RESULTS

Genotype collection over time yielded a heterogeneous distribution depending on the analyzed gene. APOE genoty-
ing consisted of 2455 samples, AGT 2067, ACE 1964, NOS3 1607, MTHFR 1114, CETP 1111, APOB, APOC3, F2, F5 and LPL 515, and IL1B, IL6, IL6R and TNF 513.

Table 2 shows demographic and clinical characteristics of participants.

Allele, genotype, and haplotype frequencies are shown in Table 3 and Fig. (2). There were no significant differences in genotype frequencies between each of the control groups, and minor allele frequencies of SNPs were in close agreement with the published data for European Caucasians (dbSNP) [32].

Deviations from Hardy-Weinberg equilibrium (HWE) were detected for LPL genotype distribution in hypercholesterolemic cases (chi-square=9.99, d.f.=2, P=0.0067), hypercholesterolemia controls, (chi-square=12.14, d.f.=2, P=0.0023), and hypertension cases (chi-square=19.63, d.f.=2, P=0.0001), with a clear infra-representation of heterozygotes. These HWE deviations occur in all genotype distributions for ACE (rs4332) and AGT (rs699) polymorphisms with P<0.01.

Pearson’s chi-square test was used to assess deviation from the null hypothesis suggesting that case and controls have the same distribution of genotype counts. With this statistical approach, only two of the nineteen genetic markers that comprise the risk panel displayed significant differences between cases and controls: APOE (chi-square=39.44, d.f.=5, P=0.0001) related to hypercholesterolemia; and, AGT (chi-square=17.06, d.f.=2, P=0.0002) related to hypertension.

Risks of hypercholesterolemia, hypertension, and cognitive impairment associated with heterozygous and homozygous variant genotypes, both individually and combined, were computed (Fig. 3 - Fig. 6).

Depending of the genetic model, risk calculation varied with considerable differences in odds ratio scores.

Regarding hypercholesterolemia, dominant genetic model assumption (Fig. 3) showed significant increased risk for CETP (odds ratio (OR), 1.22; 95% confidence interval (95% CI), 0.95-1.58), NOS3 (odds ratio (OR), 1.40; 95% confidence interval (95% CI), 1.03-1.92), TNF (odds ratio (OR), 1.37; 95% confidence interval (95% CI), 0.91-2.06), F2 (odds ratio (OR), 1.25; 95% confidence interval (95% CI), 0.45-3.49), and F5 (odds ratio (OR), 1.67; 95% confidence interval (95% CI), 0.44-6.29). In hypertensive patients, the most relevant polymorphisms were APOB (odds ratio (OR), 1.95; 95% confidence interval (95% CI), 0.63-1.33), APOC3 (odds ratio (OR), 1.51; 95% confidence interval (95% CI), 0.46-1.51), AGT174 (odds ratio (OR), 1.63; 95% confidence interval (95% CI), 0.72-1.32), TNF (odds ratio (OR), 1.23; 95% confidence interval (95% CI), 0.82-1.85), and F3 (odds ratio (OR), 1.27; 95% confidence interval (95% CI), 0.31-5.14). For dementia, the risk markers

| Gene       | dbSNP ID | Polymorphism               | TaqMan Assay ID |
|------------|----------|----------------------------|-----------------|
| APOB       | rs693    | c.7545C>T; p.Thr2515=      | C_7615420_20    |
| APOC3      | rs5128   | c.*40C>G; S1/S2            | C_8907537_1     |
| APOE       | rs429358 | c.3932T>C; Cys112Arg       | C_3084793_20    |
| APOE       | rs7412   | c.4070C>T, Cys158Arg       | C_904973_10     |
| CETP       | rs708272 | c.*279G>A                  | C_9615318_10    |
| LPL        | rs328    | c.1421C>G; p.Ser447Ter      | C_901792_1      |
| ACE        | rs4332   | c.496-66T>C                | C_11942538_20   |
| AGT        | rs4762   | c.620C>T; p.hr207Met (T174M)| C_1985480_20    |
| AGT        | rs699    | c.803T>C; p.Met268Thr (M235T)| C_1985481_20   |
| NOS3       | rs1799983| c.894T>G; p.Asp298Glu       | C_3219460_20    |
| IL1B       | rs1143634| c.315C>T; p.Phe105=        | C_9546517_10    |
| IL6        | rs1800795| c.274C>G; G-174C           | C_1839697_20    |
| IL6        | rs1800796| c.-636G>C; G-573C          | C_11326893_10   |
| IL6R       | rs2228145| c.1073A>C; p.Asp358Ala     | IL6R_1510       |
| TNF        | rs1800629| c.-488G>A; G-308A          | C_7514879_10    |
| F2         | rs1799963| c.*97G>A                   | C_8726802_20    |
| F5         | rs6025   | c.1601G>A; p.Arg534Gln     | C_11975250_10   |
| MTHFR      | rs1801133| c.665C>T; p.Ala222Val (C677T)| C_1202883_20   |
| MTHFR      | rs1801131| c.1286A>C; p.Glu429Ala (A1298C)| C_850486_20   |
Table 2. Demographic and clinical characteristics.

| Characteristics                  | Cases (mean ± SD) | Controls (mean ± SD) | Cases (mean ± SD) | Controls (mean ± SD) | Cases (mean ± SD) | Controls (mean ± SD) |
|----------------------------------|------------------|----------------------|------------------|----------------------|------------------|----------------------|
| Subjects (n)                     | 905              | 1551                 | 1311             | 1129                 | 1077             | 1268                 |
| Age (mean ± SD)                  | 66.13 ± 9.68     | 67.95 ± 10.01        | 68.89 ± 9.34     | 65.44 ± 10.22        | 72.30 ± 8.71     | 63.52 ± 8.91         |
| Gender (Male, %)                 | 35.36            | 49.32                | 45.00            | 43.31                | 34.73            | 52.13                |
| BMI (mean ± SD)                  | 28.08 ± 4.47     | 28.12 ± 4.51         | 28.68 ± 4.38     | 27.40 ± 4.52         | 27.85 ± 4.56     | 28.32 ± 4.40         |
| SBP (mean ± SD)                  | 140 ± 22         | 139 ± 21             | 155 ± 16         | 122 ± 10             | 141 ± 20         | 139 ± 22             |
| DBP (mean ± SD)                  | 81 ± 11          | 79 ± 11              | 85 ± 11          | 74 ± 7               | 79 ± 11          | 80 ± 11              |
| Total-C (mean ± SD)              | 267 ± 32         | 195 ± 28             | 223 ± 46         | 220 ± 45             | 221 ± 47         | 222 ± 45             |
| LDL-C (mean ± SD)                | 183 ± 30         | 120 ± 25             | 144 ± 41         | 142 ± 40             | 143 ± 41         | 144 ± 40             |
| HDL-C (mean ± SD)                | 59 ± 16          | 53 ± 14              | 54 ± 15          | 55 ± 15              | 55 ± 14          | 55 ± 15              |
| MMSE (mean ± SD)                 | 23 ± 7           | 23 ± 7               | 23 ± 7           | 23 ± 8               | 17 ± 6           | 28 ± 2               |

BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, Total-C: Total cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, HDL-C: High Density Lipoprotein Cholesterol, MMSE: Mini Mental State Examination.

Table 3. Allelic and genotypic frequencies of polymorphisms related to lipid metabolism.

| Gene | dbSNP ID | Polymorphism | Risk Allele | P. Value |
|------|----------|--------------|-------------|----------|
| APOB rs693 | c.7545C>T; p.Thr2515= | 0.3234 0.3075 0.4611 0.5029 0.2156 0.1897 0.4461 0.4411 | n.s. |
| APOC3 rs5128 | c*40C>G; S1/S2 | 0.8623 0.8305 0.1371 0.1523 0.0060 0.0160 0.0719 0.0934 | n.s. |
| APOE rs429358 | c.3932T>C; Cys112Arg | 0.7099 0.7198 0.2481 0.2576 0.0421 0.0226 0.1661 0.1514 | P < 0.01 |
| CETP rs708272 | c.279G>A | 0.9446 0.8909 0.0532 0.1052 0.0022 0.0039 0.0288 0.0565 | P < 0.01 |
| LPL rs328 | c.1421C>G; p.Ser447Ter | 0.6826 0.7579 0.2395 0.1988 0.0778 0.0432 0.8024 0.8573 | n.s. |

n.s.: not significant.
Fig. (2). APOE haplotype frequencies.

were APOE*4-genotypes (odds ratio (OR), 2.16; 95% confidence interval (95% CI), 1.80-2.59), LPL (odds ratio (OR), 1.43; 95% confidence interval (95% CI), 0.63-3.29), AGT235 (odds ratio (OR), 1.44; 95% confidence interval (95% CI), 1.14-1.81), and F2 (odds ratio (OR), 1.78; 95% confidence interval (95% CI), 0.65-4.86).

The recessive model assumption (Fig. 4), that only analyzed variant risk homozygotes, identified more vascular risk variants. Hypercholesterolemic genotypes were APOB*TT (odds ratio (OR), 1.17; 95% confidence interval (95% CI), 0.74-1.85), CETP*AA (odds ratio (OR), 1.21; 95% confidence interval (95% CI), 0.85-1.72), NOS3*GG (odds ratio (OR), 1.30; 95% confidence interval (95% CI), 1.06-1.61), ACE*CC (odds ratio (OR), 1.44; 95% confidence interval (95% CI), 0.91-2.30), AGT235*CC (odds ratio (OR), 0.00; 95% confidence interval (95% CI), 1.13-1.41), IL6-174*GG (odds ratio (OR), 1.25; 95% confidence interval (95% CI), 0.85-1.83), IL6-573*CC (odds ratio (OR), 1.39; 95% confidence interval (95% CI), 0.56-3.46), and MTHFR677*TT (odds ratio (OR), 1.17; 95% confidence interval (95% CI), 0.83-1.64).

For hypertension, genetic risk genotypes were APOC3*GG (odds ratio (OR), 1.59; 95% confidence interval (95% CI), 0.31-8.28), APOE*4/*4 (odds ratio (OR), 1.17; 95% confidence interval (95% CI), 0.73-1.88), AGT174*TT (odds ratio (OR), 1.74; 95% confidence interval (95% CI), 0.97-3.14), AGT235*CC (odds ratio (OR), 1.26; 95% confidence interval (95% CI), 1.01-1.57), IL1B*TT (odds ratio (OR), 1.14; 95% confidence interval (95% CI), 0.49-2.63), TNF*AA (odds ratio (OR), 1.15; 95% confidence interval
Fig. (3). Odds ratio (OR) results from hypercholesterolemia, hypertension, and dementia: Dominant model (homozygote + heterozygote risk allele).
Fig. (4). Odds ratio (OR) results from hypercholesterolemia, hypertension, and dementia: Recessive model (homozygote risk allele).
Fig. (5). Odds ratio (OR) results from hypercholesterolemia, hypertension, and dementia: Multiplicative genetic model (allele counts).
(95% CI), 0.38-3.48), and MTHFR677*TT (odds ratio (OR), 1.35; 95% confidence interval (95% CI), 0.95-1.92).

In demented patients, APOE*4/*4 (odds ratio (OR), 2.53; 95% confidence interval (95% CI), 1.53-4.18), AGT235*CC (odds ratio (OR), 1.16; 95% confidence interval (95% CI), 0.93-1.45), IL6R*CC (odds ratio (OR), 1.19; 95% confidence interval (95% CI), 0.73-1.93), and MTHFR677*TT genotypes (odds ratio (OR), 1.13; 95% confidence interval (95% CI), 0.80-1.58) were significantly overrepresented.

The multiplicative genetic model (Fig. 5) quantified the risk for allele presence or absence in hypercholesterolemic patients: APOC3*G (odds ratio (OR), 1.33; 95% confidence interval (95% CI), 0.82-2.17) and APOE*4 (odds ratio (OR), 1.12; 95% confidence interval (95% CI), 0.95-1.31), AGT174*T (odds ratio (OR), 1.28; 95% confidence interval (95% CI), 1.06-1.56), IL1B*T (odds ratio (OR), 1.15; 95% confidence interval (95% CI), 0.83-1.60), and IL6R*C (odds ratio (OR), 1.17; 95% confidence interval (95% CI), 0.89-1.52); hypertensive patients: APOE*4 (odds ratio (OR), 1.15; 95% confidence interval (95% CI), 0.98-1.34), IL1B*T (odds ratio (OR), 1.12; 95% confidence interval (95% CI), 0.82-1.53), IL6-573*C (odds ratio (OR), 1.74; 95% confidence interval (95% CI), 1.19-2.54), F2*A (odds ratio (OR), 1.59; 95% confidence interval (95% CI), 0.59-4.28), and MTHFR1298*C (odds ratio (OR), 1.26; 95% confidence interval (95% CI), 0.95-1.65).
## Table 4. Allelic and genotypic frequencies of polymorphisms related to endothelial function and hypertension.

| Gene   | dbsNP ID | Polymorphism | Genotypes | Risk Allele | P. values |
|--------|----------|--------------|-----------|-------------|-----------|
|        |          |              | Cases     | Controls    | Cases     | Controls    | Cases     | Controls    |        |
|        |          |              | TT        | TC          | CC        | C          |          |             |        |
| ACE    | rs4332   | c.496-66T>C  |            |             |           |            |           |             | C          |
|        |          |              | Hypercholesterolemia | 0.4795 | 0.4873 | 0.3099 | 0.3569 | 0.2105 | 0.1558 | 0.3655 | 0.3343 | n.s. |
|        |          |              | Hypertension  | 0.4780 | 0.4901 | 0.3459 | 0.3416 | 0.1761 | 0.1683 | 0.3491 | 0.3391 | n.s. |
|        |          |              | Dementia     | 0.4571 | 0.5053 | 0.3714 | 0.3333 | 0.1714 | 0.1614 | 0.3571 | 0.3281 | n.s. |
| AGT    | rs4762   | c.620C>T; p.hr207Met (T174M) | CC       | CT          | TT        | T          |          |             |        |
|        |          |              | Hypercholesterolemia | 0.7877 | 0.7463 | 0.1966 | 0.2237 | 0.0157 | 0.0300 | 0.1140 | 0.1418 | P < 0.05 |
|        |          |              | Hypertension  | 0.7443 | 0.7798 | 0.2248 | 0.2023 | 0.0308 | 0.0179 | 0.1432 | 0.1191 | P < 0.05 |
|        |          |              | Dementia     | 0.7627 | 0.7597 | 0.2215 | 0.2114 | 0.0158 | 0.0289 | 0.1266 | 0.1346 | n.s. |
| AGT    | rs699    | c.803T>C; p.Met268Thr (M235T) | TT        | TC          | CC        | C          |          |             |        |
|        |          |              | Hypercholesterolemia | 0.1769 | 0.1945 | 0.6081 | 0.6111 | 0.2149 | 0.1945 | 0.5190 | 0.5000 | n.s. |
|        |          |              | Hypertension  | 0.2112 | 0.1602 | 0.5694 | 0.6575 | 0.2194 | 0.1823 | 0.5041 | 0.5111 | n.s. |
|        |          |              | Dementia     | 0.1559 | 0.2096 | 0.6271 | 0.5980 | 0.2169 | 0.1924 | 0.5305 | 0.4972 | n.s. |
| NOS3   | rs1799983| c.894T>G; p.Asp298Glu | TT        | TG          | GG        | G          |          |             |        |
|        |          |              | Hypercholesterolemia | 0.1073 | 0.1444 | 0.4753 | 0.5010 | 0.4174 | 0.3546 | 0.6550 | 0.6051 | n.s. |
|        |          |              | Hypertension  | 0.1371 | 0.1232 | 0.4872 | 0.4943 | 0.3757 | 0.3825 | 0.6193 | 0.6297 | n.s. |
|        |          |              | Dementia     | 0.1424 | 0.1220 | 0.4864 | 0.4927 | 0.3712 | 0.3853 | 0.6144 | 0.6316 | n.s. |

n.s.: not significant.

When calculating odds ratios (ORs) separately for variant risk homozygotes and heterozygotes (Fig. 6), the most informative polymorphisms related to risk for hypercholesterolemia were APOC3*G (odds ratio (OR), 1.40; 95% confidence interval (95% CI), 0.88-2.23), APOE*4 (odds ratio (OR), 1.99; 95% confidence interval (95% CI), 1.70-2.34), IL6-174*G (odds ratio (OR), 1.11; 95% confidence interval (95% CI), 0.86-1.44), IL6-573*C (odds ratio (OR), 1.12; 95% confidence interval (95% CI), 0.76-1.66), and F5*4 (odds ratio (OR), 1.48; 95% confidence interval (95% CI), 0.37-5.95).

For hypertension, relevant genotypes were APOB*CT (odds ratio (OR), 1.42; 95% confidence interval (95% CI), 0.95-2.14), APOB*TT (odds ratio (OR), 1.13; 95% confidence interval (95% CI), 0.68-1.89), APOC3*CG (odds ratio (OR), 1.49; 95% confidence interval (95% CI), 0.88-2.53), APOC3*GG (odds ratio (OR), 1.68; 95% confidence interval (95% CI), 0.32-8.77), APOE*3/*4 (odds ratio (OR), 1.16; 95% confidence interval (95% CI), 0.96-1.41), APOE*4/*4 (odds ratio (OR), 1.21; 95% confidence interval (95% CI), 0.75-1.94), LPL*GG (odds ratio (OR), 1.63; 95% confidence interval (95% CI), 0.70-3.80), ACE*CC (odds ratio (OR), 1.54; 95% confidence interval (95% CI), 0.70-1.04), AGT174*CT (odds ratio (OR), 1.16; 95% confidence interval (95% CI), 0.92-1.58), AGT235*CC (odds ratio (OR), 1.21; 95% confidence interval (95% CI), 0.91-1.62), IL6-373*CC (odds ratio (OR), 1.39; 95% confidence interval (95% CI), 0.56-3.49), TNF*GA (odds ratio (OR), 1.40; 95% confidence interval (95% CI), 0.92-2.15), F2*GA (odds ratio (OR), 1.25; 95% confidence interval (95% CI), 0.45-3.49), F5*GA (odds ratio (OR), 1.07; 95% confidence interval (95% CI), 0.44-6.29), and MTHFR677*TT (odds ratio (OR), 1.19; 95% confidence interval (95% CI), 0.82-1.73).
Table 5. Allelic and genotypic frequencies of polymorphisms related to immune response and inflammation.

| Gene   | dbsNP ID  | Polymorphism                      | Genotypes | Risk Allele | P. value |
|--------|-----------|-----------------------------------|-----------|-------------|----------|
|        |           |                                   | Cases     | Controls    | Cases     | Controls    | Cases     | Controls    |         |
|        |           |                                   | CC        | CT          | TT        | T          |          |             |         |
|        | IL1B      | c.315C>T; p.Phe105=                |           |             |           |            |          |             |         |
|        | Hypercholesterolemia | 0.6548 | 0.6290 | 0.3095 | 0.3159 | 0.0357 | 0.0551 | 0.1905 | 0.2130 | n.s. |
|        | IL6       | c.-274C>G; G-174C                 | CC        | CG          | GG        | G          |          |             |         |
|        | Hypercholesterolemia | 0.2143 | 0.1797 | 0.4048 | 0.4899 | 0.3810 | 0.3304 | 0.5833 | 0.5754 | n.s. |
|        |          |                                   |           |             |           |            |          |             |         |
|        |          | IL6       | c.-363G>C; G-573C                 | GG        | GC          | CC        | C          |          |             |         |
|        | Hypercholesterolemia | 0.7857 | 0.8000 | 0.1667 | 0.1652 | 0.0476 | 0.0348 | 0.1310 | 0.1174 | n.s. |
|        |          |          | IL6R      | c.1073A>C; p.Asp358Ala | AA        | AC          | CC        | C          |          |         |
|        | Hypercholesterolemia | 0.3810 | 0.3420 | 0.4821 | 0.4870 | 0.1369 | 0.1710 | 0.3780 | 0.4145 | n.s. |
|        |          |          |          |             |           |            |          |             |         |
|        |          | TNF      | c.-488G>A; G-308A                  | GG        | GA          | AA        | A          |          |             |         |
|        | Hypercholesterolemia | 0.6905 | 0.7536 | 0.2798 | 0.2174 | 0.0298 | 0.0290 | 0.1696 | 0.1377 | n.s. |
|        |          |          |          |             |           |            |          |             |         |

n.s.: not significant.

(95% CI, 0.94-1.44), AGT174*TT (odds ratio (OR), 1.80; 95% confidence interval (95% CI), 1.00-3.25), IL6-174*CG (odds ratio (OR), 1.24; 95% confidence interval (95% CI), 0.76-2.01), TNF*GA (odds ratio (OR), 1.24; 95% confidence interval (95% CI), 0.81-1.89), TNF*AA (odds ratio (OR), 1.21; 95% confidence interval (95% CI), 0.40-3.68), F5*GA (odds ratio (OR), 1.27; 95% confidence interval (95% CI), 0.31-5.14), and MTHFR677*TT (odds ratio (OR), 1.24; 95% confidence interval (95% CI), 0.85-1.82).

Demented patients showed the following genetic risk profile: APOE*3*/4 (odds ratio (OR), 2.06; 95% confidence interval (95% CI), 1.70-2.51), APOE*4*/4 (odds ratio (OR), 3.08; 95% confidence interval (95% CI), 1.85-5.12), LPL*CG (odds ratio (OR), 1.14; 95% confidence interval (95% CI), 0.73-1.77), ACE*TC (odds ratio (OR), 1.14; 95% confidence interval (95% CI), 0.73-1.77), ACE*CC (odds ratio (OR), 1.23; 95% confidence interval (95% CI), 0.83-1.83), AGT235*TC (odds ratio (OR), 1.41; 95% confidence interval (95% CI), 1.11-1.79), AGT235*CC (odds ratio (OR), 1.52; 95% confidence interval (95% CI), 1.14-2.02), IL6R*CC (odds ratio (OR), 1.16; 95% confidence interval (95% CI), 0.68-1.99), F2*GA (odds ratio (OR), 1.78; 95% confidence interval (95% CI), 0.65-4.86), and MTHFR677*TT (odds ratio (OR), 1.17; 95% confidence interval (95% CI), 0.81-1.70).

4. DISCUSSION

Different variations affecting the amino acid sequence (exonic polymorphisms) or the level of genetic expression (promoter region polymorphisms) of genes encoding apolipoproteins have been observed in several studies [6-10]. These genetic variations are related to total cholesterol,
low-density lipoproteins, triglycerides, and vascular disorders [33, 34]. Depending on the genetic model assumed, we could identify the relevance of these polymorphisms related to hypercholesterolemia and vascular risk in a different manner. APOB*TT homozygotes (Fig. 3b), and genotypes including APOC3*G and APOE*4 alleles (Fig. 3c) showed a moderately increased risk for hypercholesterolemic patients when compared with normolipidemic subjects. The odds ratio calculation assuming the APOE*3/*3 genotype as the no risk genotype (OR = 1) revealed a highly increased risk for APOE*4/*4 (odds ratio (OR), 1.75; 95% confidence interval (95% CI), 1.09-2.80) (Fig. 4).

The LPL 1421C>G polymorphism showed increased risk related to hypercholesterolemia in both heterozygous and homozygous patients for the allelic risk variants LPL CG and GG (Fig. 4). These results are in accordance with previously reported studies linking LPL polymorphisms with lipid plasma levels and the prevalence of coronary artery disease in non-protective LPL*G variant carriers [12, 13].

The results of CETP +279G>A (TaqI polymorphism) are controversial. Previous studies showed a strong association between the TaqI B2 allele (CETP*+279G), high HDL-cholesterol levels and reduced risk of vascular disorders [11, 32], but in others, a relationship between the B2 variant and cardiovascular disorders (i.e. atrial fibrillation) has been reported [35, 36].

The contribution of ACE, NOS3 and AGT polymorphisms to hypertension has been established in different studies [14-18]. ACE, a dipeptidyl carboxypeptidase that plays an important role in regulating blood pressure and electrolyte balance, hydrolyses angiotensin I to angiotensin II; a potent vasopressor and aldosterone-stimulating peptide. The enzyme is also capable of inactivating bradykinin; a potent vasodilator. ACE mutations are associated with a high predisposition to develop essential hypertension, which predisposes to suffering other cardiovascular diseases [15, 16]. The AGT gene encodes angiotensinogen, which is converted to angiotensin I by renin. 235T and 174M alleles are associated with an increased risk of essential hypertension [17, 18]. Our results support the role of angiotensinogen in the development of hypertension with OR values above 1.5 for ACE*CC and AGT174*TT homozygotes (Fig. 3 and Fig. 4).

At present, the important role of inflammation in the initiation and progression of atherosclerosis is well known [2] and it is suspected that inflammation plays a role in the triggering of the thrombotic coagulation process [25].
flammatory cytokine polymorphisms have been postulated to modulate the inflammatory pattern involved in forming clots that could trigger arterial ischemic processes [19], increased levels of plasma triglycerides, VLDL and free fatty acids, as well as lower levels of HDL-cholesterol [20-26]. Our results show the importance of IL6 and TNF polymorphisms as the markers for increased risk of hypercholesterolemia, hypertension and vascular disorders (Fig. 4).

The genetic risk profile tested in our study shows the relevance of significant dementia-related polymorphisms such as APOE*4, ACE*C, and AGT235*C. Especially relevant is the case of APOE, where heterozygous APOE*3/4 and homozygous APOE*4/*4 have, respectively, 2-fold and 3-fold greater risk for developing dementia than non-carriers of the APOE*4 allele.

Since pathogenic genes are determinant in the therapeutic outcome associated with the pharmacogenomics of dementia and cerebrovascular disorders [37-40], most polymorphic risk variants identified in this study can also be used as potential markers for drug efficacy and safety in pharmacogenetic studies [41, 42].

CONCLUSION

The results derived from the cerebrovascular genetic risk test indicate that a person may have a greater probability, risk or susceptibility for suffering hypercholesterolemia, hypertension, or cognitive impairment that are related to the final irruption of the cerebrovascular disease than in the population at large. The multifactorial nature of this pathology requires the development of panels of genetic markers that allow us to know the negative load of risk of the patient and that help us to determine the moment of onset, evolution and prognosis of the disease in order to be able to choose the appropriate treatment in each particular case.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Relevance of Genetic Risk Factors in the Management of Cerebrovascular Disorders

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