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RESEARCH ARTICLE

Genetic Risk Factors in Cerebrovascular Disorders and Cognitive Deterioration

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ARTICLE HISTORY

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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 foam 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 vey 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].

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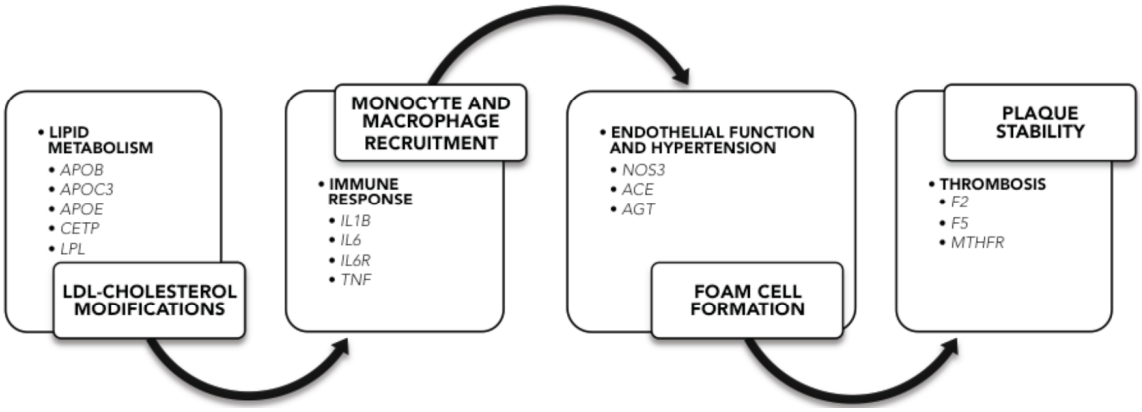

Fig. (1). Genes involved in the atherogenic process.

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* genotyp-

Table 1. Genetic polymorphisms involved in the present study.

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

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 *F5* (odds ratio (OR), 1.27; 95% confidence interval (95% CI), 0.31-5.14). For dementia, the risk markers

Table 2. Demographic and clinical characteristics.

——	Hypercholesterolemia		Hypertension		Cognitive Impairment	
Characteristics	**Cases**	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**
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	Genotypes						Risk Allele		P. Value
–	–	–	**Cases**	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**	–
APOB	rs693	c.7545C>T; p.Thr2515=	CC		CT		TT		T		
		Hypercholesterolemia	0.3234	0.3075	0.4611	0.5029	0.2156	0.1897	0.4461	0.4411	n.s.
		Hypertension	0.2907	0.3535	0.5208	0.4444	0.1885	0.2020	0.4489	0.4242	n.s.
		Dementia	0.3510	0.2857	0.4471	0.5286	0.2019	0.1857	0.4255	0.4500	n.s.
APOC3	rs5128	c*40C>G; S1/S2	CC		CG		GG		G		–
		Hypercholesterolemia	0.8623	0.8305	0.1371	0.1523	0.0060	0.0160	0.0719	0.0934	n.s.
		Hypertension	0.8211	0.8737	0.1629	0.1162	0.0160	0.0101	0.0974	0.0682	n.s.
		Dementia	0.8558	0.8286	0.1442	0.1464	0.0000	0.0250	0.0721	0.0982	n.s.
APOE	rs429358	c.3932T>C; Cys112Arg	TT		TC		CC		C		–
		Hypercholesterolemia	0.7099	0.7198	0.2481	0.2576	0.0421	0.0226	0.1661	0.1514	P < 0.01
		Hypertension	0.7018	0.7340	0.2661	0.2385	0.0321	0.0275	0.1651	0.1467	n.s.
		Dementia	0.6298	0.7859	0.3256	0.1959	0.0447	0.0182	0.2074	0.1161	n.s.
APOE	rs7412	c.4070C>T. Cys158Arg	CC		CT		TT		T		–
		Hypercholesterolemia	0.9446	0.8909	0.0532	0.1052	0.0022	0.0039	0.0288	0.0565	P < 0.01
		Hypertension	0.9174	0.9043	0.0810	0.0904	0.0015	0.0053	0.0420	0.0505	n.s.
		Dementia	0.9126	0.9076	0.0847	0.0893	0.0028	0.0032	0.0451	0.0478	n.s.
CETP	rs708272	c.+279G>A	GG		GA		AA		A		–
		Hypercholesterolemia	0.3384	0.3846	0.5101	0.4867	0.1515	0.1287	0.4066	0.3720	n.s.
		Hypertension	0.3775	0.3612	0.4855	0.5079	0.1370	0.1309	0.3798	0.3849	n.s.
		Dementia	0.3714	0.3696	0.4967	0.4916	0.1319	0.1388	0.3802	0.3846	n.s.
LPL	rs328	c.1421C>G; p.Ser447Ter	CC		CG		GG		C		–
		Hypercholesterolemia	0.6826	0.7579	0.2395	0.1988	0.0778	0.0432	0.8024	0.8573	n.s.
		Hypertension	0.7252	0.7513	0.2109	0.2081	0.0639	0.0406	0.8307	0.8553	n.s.
		Dementia	0.7308	0.7384	0.2260	0.2007	0.0433	0.0609	0.8387	0.9188	P < 0.05

n.s.: not significant.

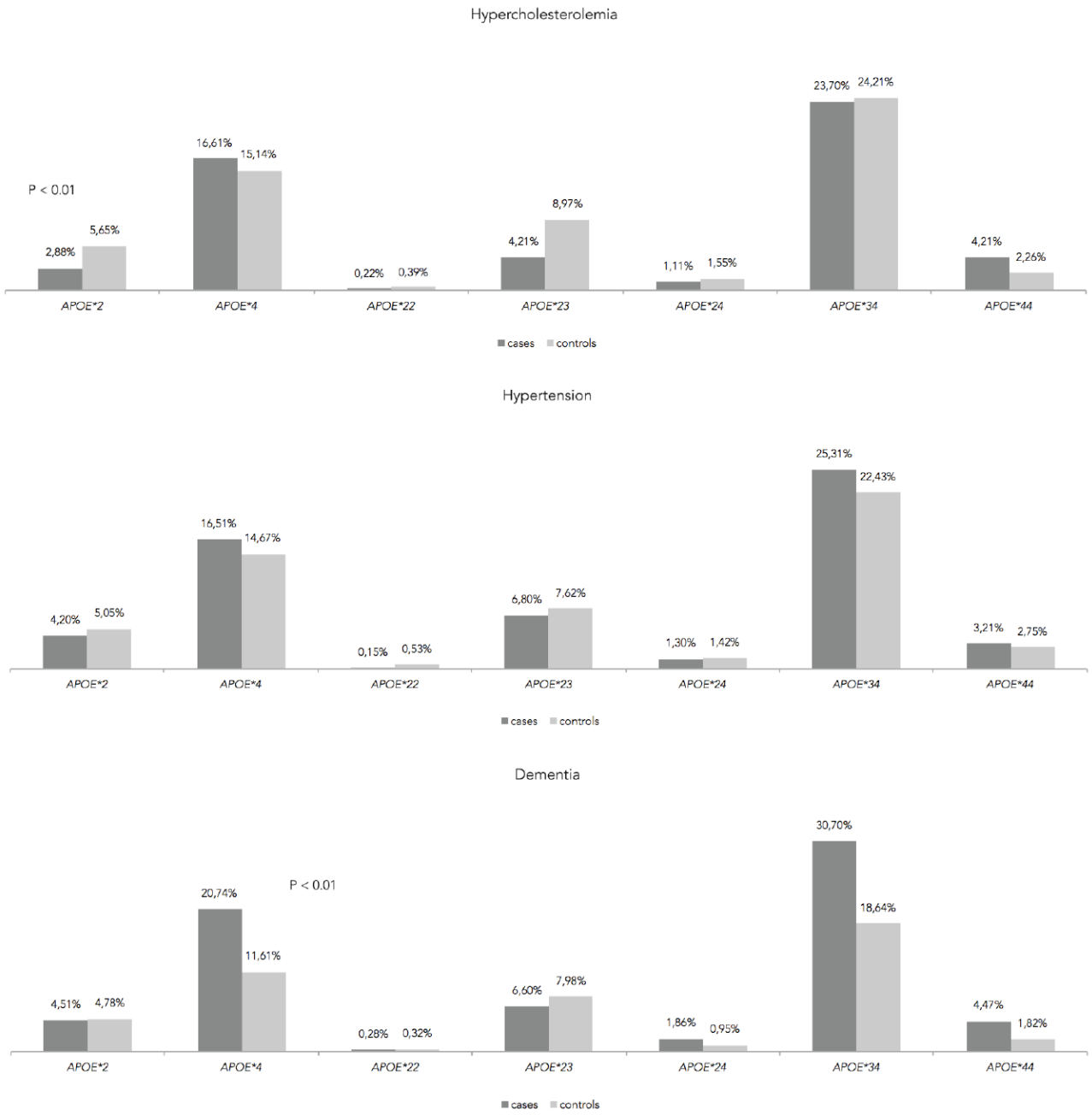

Fig. (2). *APOE* haplotype frequencies.

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), *IL1B1*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

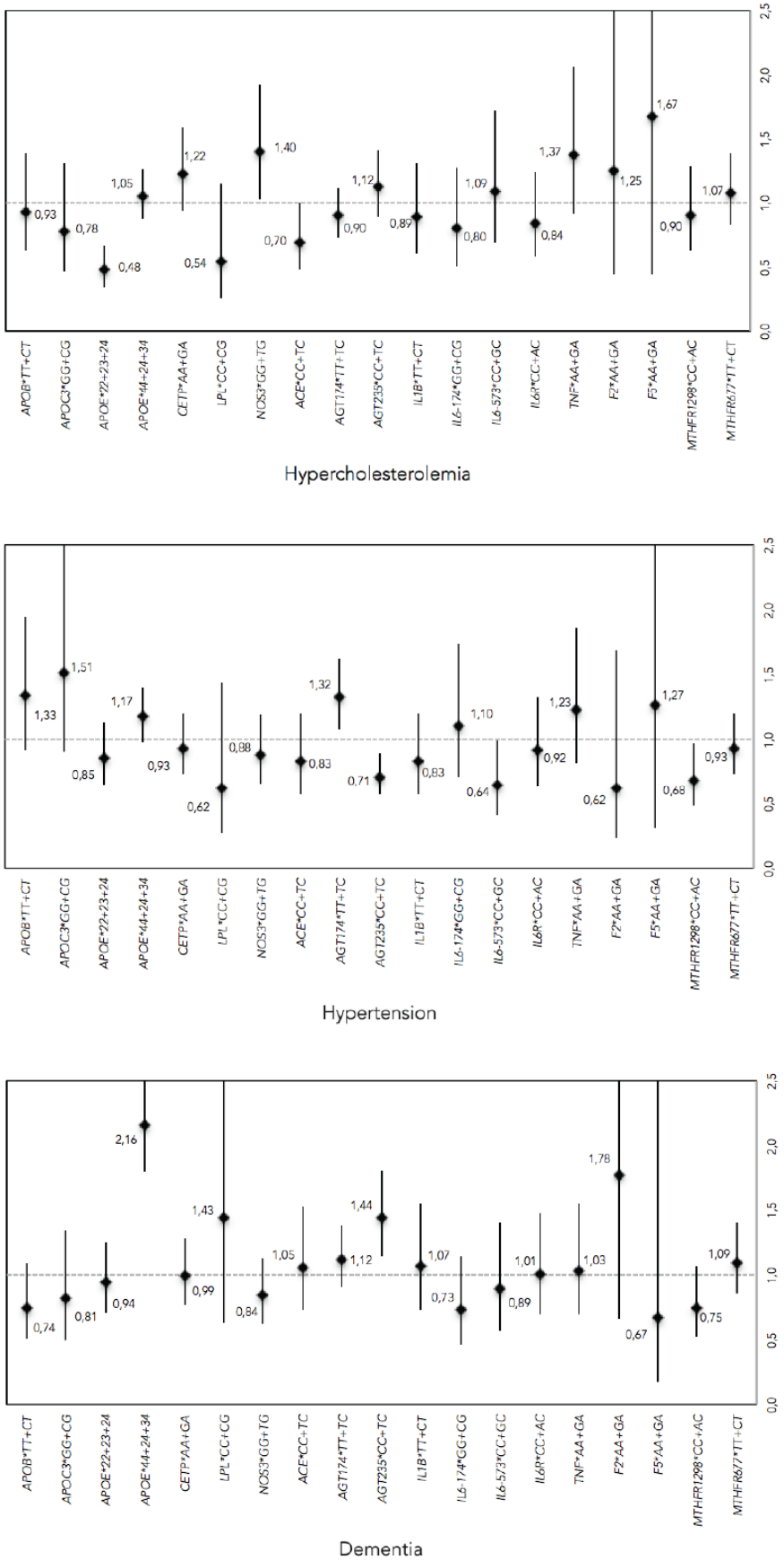

Fig. (3). Odds ratio (OR) results from hypercholesterolemia, hypertension, and dementia: Dominant model (homozygote + heterozygote risk allele).

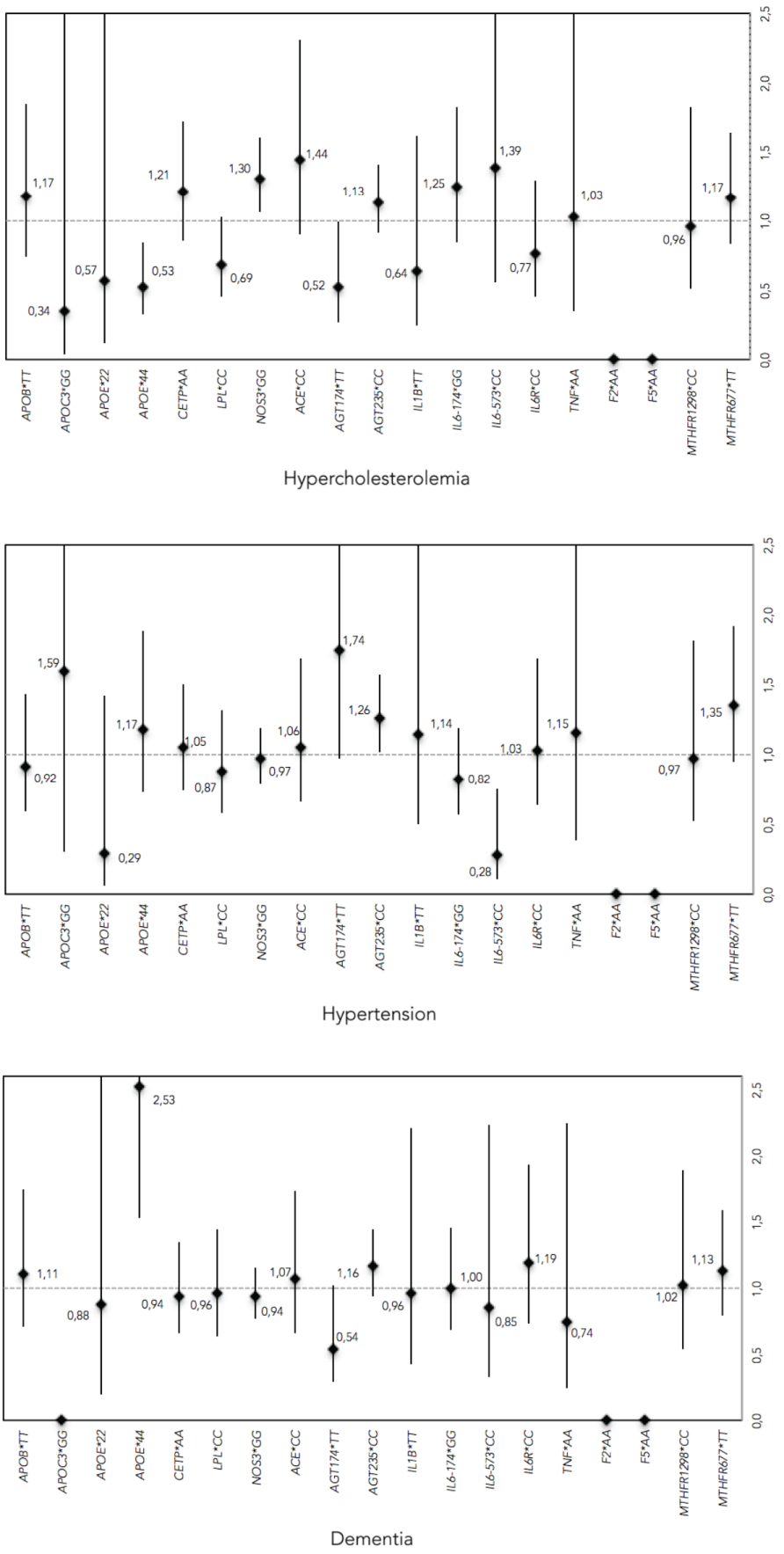

Fig. (4). Odds ratio (OR) results from hypercholesterolemia, hypertension, and dementia: Recessive model (homozygote risk allele).

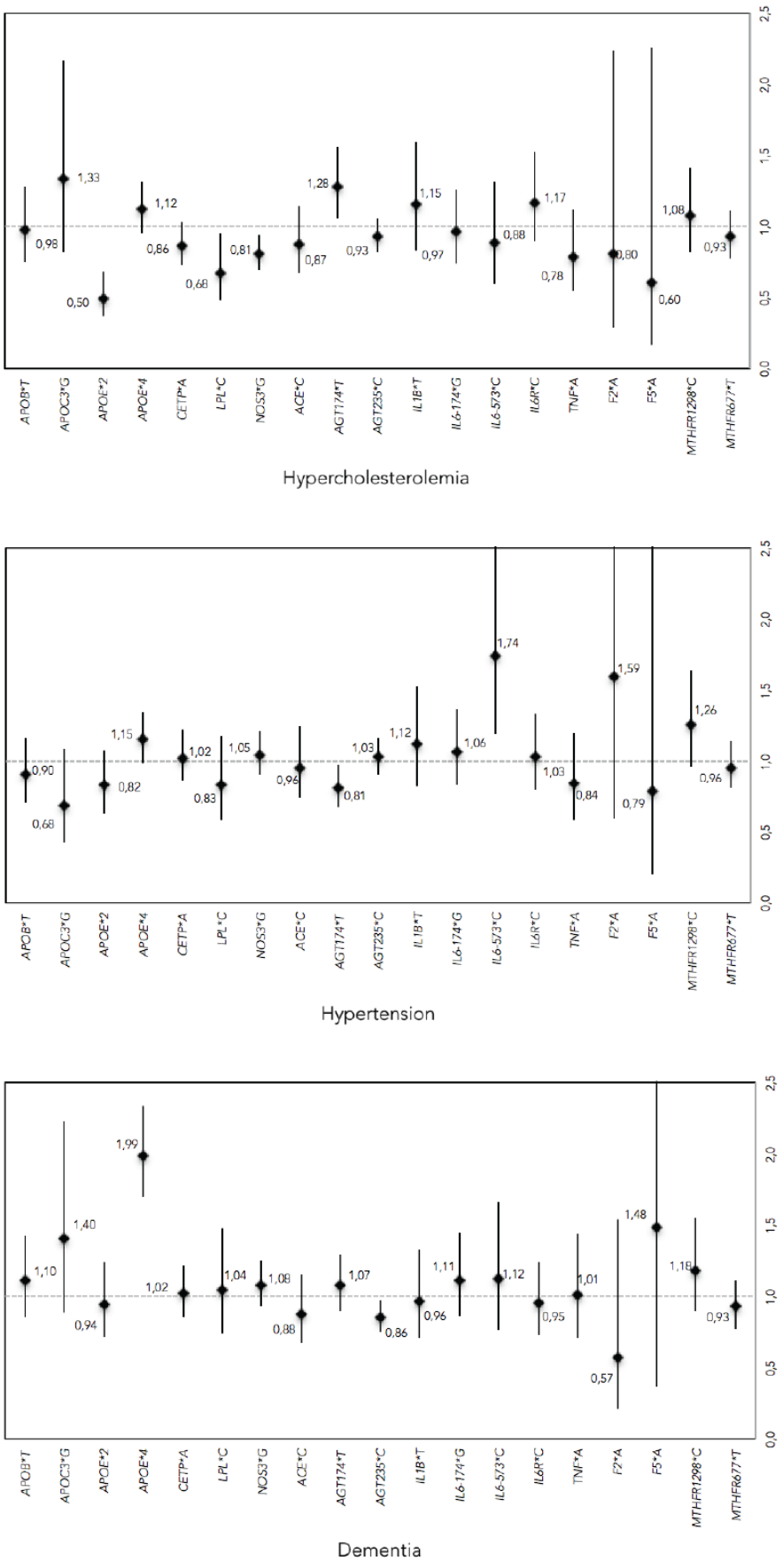

Fig. (5). Odds ratio (OR) results from hypercholesterolemia, hypertension, and dementia: Multiplicative genetic model (allele counts).

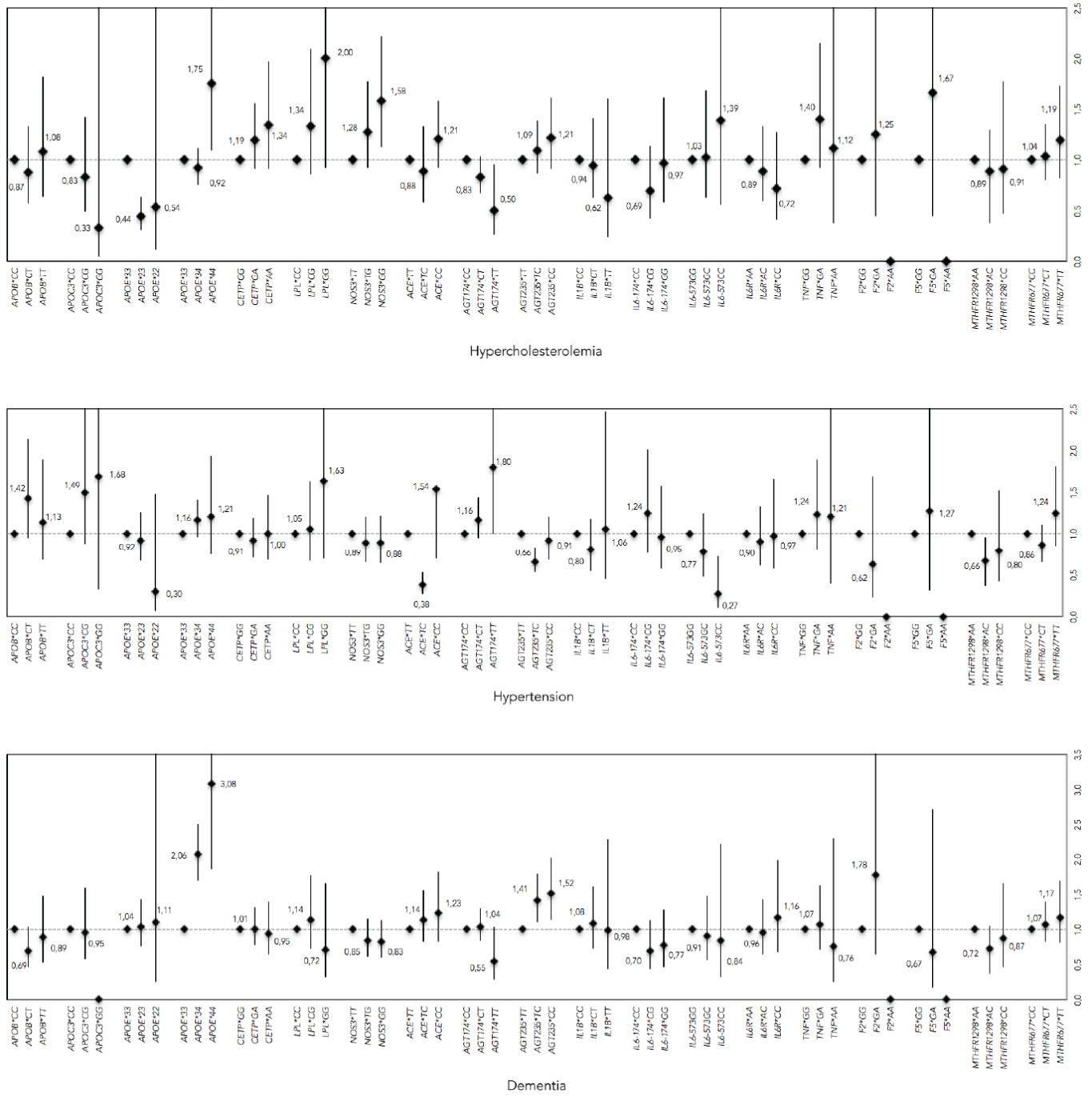

Fig. (6). Homozygote and heterozygote risk calculation (OR) comparing each to the baseline no-risk homozygote (OR=1).

(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

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	Cases	Controls	–
ACE	rs4332	c.496-66T>C	TT		TC		CC		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.

interval (95% CI), 0.96-1.64); and demented patients: *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*A* (odds ratio (OR), 1.48; 95% confidence interval (95% CI), 0.37-5.95).

When calculating odds ratios (ORs) separately for variant risk homozygotes and heterozygotes (Fig. **6**), the most informative polymorphisms related to risk for hypercholesterolemia were *APOE*4/*4* (odds ratio (OR), 1.75; 95% confidence interval (95% CI), 1.09-2.80), *CETP*GA* (odds ratio (OR), 1.19; 95% confidence interval (95% CI), 0.91-1.56), *CETP*AA* (odds ratio (OR), 1.34; 95% confidence interval (95% CI), 0.91-1.97), *LPL*CG* (odds ratio (OR), 1.34; 95% confidence interval (95% CI), 0.86-2.09), *LPL*GG* (odds ratio (OR), 2.00; 95% confidence interval (95% CI), 0.92-4.34), *NOS3*TG* (odds ratio (OR), 1.28; 95% confidence interval (95% CI), 0.92-1.77), *NOS3*GG* (odds ratio (OR), 1.58; 95% confidence interval (95% CI), 1.13-2.22), *ACE*CC* (odds ratio (OR), 1.21; 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), *IL6-573*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), 1.67; 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).

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

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	Cases	Controls	–
IL1B	rs1143634	c.315C>T; p.Phe105=	CC		CT		TT		T		
		Hypercholesterolemia	0.6548	0.6290	0.3095	0.3159	0.0357	0.0551	0.1905	0.2130	n.s.
		Hypertension	0.6592	0.6162	0.2894	0.3384	0.0514	0.0455	0.1961	0.2146	n.s.
		Dementia	0.6377	0.6523	0.3140	0.2975	0.0483	0.0502	0.2053	0.1989	n.s.
IL6	rs1800795	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.
		Hypertension	0.1865	0.2020	0.4855	0.4242	0.3280	0.3737	0.5707	0.5859	n.s.
		Dementia	0.2222	0.1720	0.4348	0.4839	0.3430	0.3441	0.5604	0.5860	n.s.
IL6	rs1800796	c.-636G>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.
		Hypertension	0.8264	0.7525	0.1543	0.1818	0.0193	0.0657	0.0965	0.1566	P < 0.01
		Dementia									n.s.
IL6R	rs2228145	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.
		Hypertension	0.3633	0.3434	0.4759	0.5000	0.1608	0.1566	0.3987	0.4066	n.s.
		Dementia	0.3527	0.3548	0.4734	0.4946	0.1739	0.1505	0.4106	0.3978	n.s.
TNF	rs1800629	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.
		Hypertension	0.7170	0.7576	0.2540	0.2172	0.0289	0.0253	0.1559	0.1338	n.s.
		Dementia	0.7246	0.7312	0.2512	0.2366	0.0242	0.0323	0.1498	0.1505	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,

Table 6. Allelic and genotypic frequencies of polymorphisms related to thrombosis.

Gene	dbSNP ID	Polymorphism	Genotypes						Risk Allele		P. value
–	–	–	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	–
F2	rs1799963	c*97G>A	GG		GA		AA		A		
		Hypercholesterolemia	0.9643	0.9712	0.0357	0.0288	0.0000	0.0000	0.0179	0.0144	n.s.
		Hypertension	0.9744	0.9596	0.0256	0.0404	0.0000	0.0000	0.0128	0.0202	n.s.
		Dementia	0.9565	0.9751	0.0435	0.0249	0.0000	0.0000	0.0217	0.0125	n.s.
F5	rs6025	c.1601G>A; p.Arg534Gln	GG		GA		AA		A		–
		Hypercholesterolemia	0.9762	0.9856	0.0238	0.0144	0.0000	0.0000	0.0119	0.0072	n.s.
		Hypertension	0.9808	0.9848	0.0192	0.0152	0.0000	0.0000	0.0096	0.0076	n.s.
		Dementia	0.9855	0.9786	0.0145	0.0214	0.0000	0.0000	0.0072	0.0107	n.s.
MTHFR	rs1801133	c.665C>T; p.Ala222Val	CC		CT		TT		T		–
		Hypercholesterolemia	0.3721	0.3884	0.4677	0.4711	0.1602	0.1405	0.3941	0.3760	n.s.
		Hypertension	0.3885	0.3725	0.4507	0.5034	0.1608	0.1242	0.3862	0.3758	n.s.
		Dementia	0.3753	0.3967	0.4680	0.4617	0.1567	0.1417	0.3907	0.3725	n.s.
MTHFR	rs1801131	c.1286A>C; p.Glu429Ala	AA		AC		CC		C		–
		Hypercholesterolemia	0.4972	0.4698	0.4181	0.4423	0.0847	0.0879	0.2938	0.3091	n.s.
		Hypertension	0.5152	0.4203	0.4000	0.4928	0.0848	0.0870	0.2848	0.3333	n.s.
		Dementia	0.5117	0.4392	0.3991	0.4730	0.0892	0.0878	0.2887	0.3243	n.s.

n.s.: not significant.

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]. Proin-

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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