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RESEARCH ARTICLE

Population-based Study of Risk Polymorphisms Associated with Vascular Disorders and Dementia

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Abstract: ***Introduction*:** Cardiovascular and neurodegenerative disorders are among the major causes of mortality in the developed countries. Population studies evaluate the genetic risk, *i.e.* the probability of an individual carrying a specific disease-associated polymorphism. Identification of risk polymorphisms is essential for an accurate diagnosis or prognosis of a number of pathologies.

***Aims*:** The aim of this study was to characterize the influence of risk polymorphisms associated with lipid metabolism, hypertension, thrombosis, and dementia, in a large population of Spanish individuals affected by a variety of brain and vascular disorders as well as metabolic syndrome.

***Material & Method*:** We performed a cross-sectional study on 4415 individuals from a widespread regional distribution in Spain (48.15% males and 51.85% females), with mental, neurodegenerative, cerebrovascular, and metabolic disorders. We evaluated polymorphisms in 20 genes involved in obesity, vascular and cardiovascular risk, and dementia in our population and compared it with representative Spanish and European populations. Risk polymorphisms in *ACE*, *AGT(235)*, *IL6(573)*, *PSEN1*, and *APOE* (specially the *APOE-ε4* allele) are representative of our population as compared to the reference data of Spanish and European individuals.

***Conclusion*:** The significantly higher distribution of risk polymorphisms in *PSEN1* and *APOE-ε4* is characteristic of a representative number of patients with Alzheimer's disease; whereas polymorphisms in *ACE*, *AGT(235)*, and *IL6(573)*, are most probably related with the high number of patients with metabolic syndrome or cerebrovascular damage.

Keywords: Dementia, Alzheimer's disease, Hypertension, Metabolic syndrome, APOE, Vascular risk.

1. INTRODUCTION

Vascular and neurodegenerative disorders are among the most prevalent causes of death in Western Countries. The knowledge of the human genome allows the detection of modifications in the sequence of certain genes responsible for a number of these diseases. The appropriate genetic counseling relies on the characterization of these risk polymorphisms for accurate individual or family decision making.

Metabolic Syndrome (MS) affects 20-34% of the population [1, 2], primarily in the developed countries. Definition of MS slightly differs depending on the source [3-5], although it is generally defined by the confluence of several medical conditions, including low high-density lipoprotein (HDL) levels, high blood serum triglycerides, high blood pressure, abdominal obesity, and elevated fasting plasma glucose. Individuals with MS are more likely candidates to develop cerebrovascular and cardiovascular disease and diabetes than the general population.

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Cerebrovascular disorders and stroke are the third leading cause of death in the US and in Europe with around 200 cases per 100,000 inhabitants per year [6] and almost six million victims every year, according to the World Health Organization [7]. Cerebrovascular disorders are characterized by the blockade of arterial vascularization (atherosclerosis) with the consequent deprivation of oxygen and glucose supply to the affected tissue, leading to cell death and tissue necrosis or ischemia. Depending on the anatomical site of the ischemic insult, cerebral stroke can lead to hemiplegia, aphasia, motor dysfunction, or dementia. Atherosclerosis is a form of chronic inflammation resulting from the interaction of different factors, including enhanced uptake of low-density lipoprotein (LDL) by monocytes and macrophages [8, 9], immune response, and development of plaque deposits in the lumen of the blood vessels [10, 11]. Several genetic risk markers involved in the atherosclerosis-related pathways have been identified [12].

Certain polymorphisms in genes encoding apolipoproteins (*APOB, APOC3, APOE*) and the cholesterol ester transfer protein (*CTEP*) are associated with an aberrant increase in LDL-family lipoproteins, cholesterol, and triglyceride levels. Therefore, these gene variants are involved in the risk for atherosclerosis and vascular disorders [13-20]. However, the polymorphism C1421G (rs328) in the *LPL* gene, coding

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for the lipoprotein lipase, develops a protective effect by increasing the levels of high density lipoproteins (HDL) and decreasing the triglycerides [13, 21, 22]. One of the signs of the progression from the initial fatty streak to the more complex lesions in the artery wall is the high blood pressure. Specific polymorphisms in *NOS3, ACE*, and *AGT*, involved in endothelial function and regulation of blood pressure, are indicative of the progression of the atherosclerotic plaque [13, 23-27]. The role of inflammation in atherosclerosis is well established [11], as well as its implication in thrombosis by enhancing the coagulation process [28]. In this regard, polymorphisms in genes encoding proinflammatory interleukins, especially *IL1* and *IL6*, promote their expression leading to atherothrombosis and lacunar infarction [13, 29-34]. The variant -308G>A (rs1800629) in the tumor necrosis factor gene (*TNFA*) has also been observed to be associated with migraine, which suggests the role of this polymorphism in the regulation of blood flow throughout the brain [35]. Although the progressive narrowing of the blood vessel during the atherosclerotic process can develop ischemic symptoms, stroke is normally promoted by the rupture of the sclerotic plaque and thrombosis. Polymorphisms in the genes coding the coagulation factors F2 and F5 are associated with the accumulation of prothrombin, increasing the risk of thrombosis [13, 36-38]. The *MTHFR* gene encodes for the methylentetrahydrofolate reductase which remethylates homocysteine into methionine. The polymorphisms 1298A>C (rs1801131) and 677C>T (rs1801133) in *MTHFR* result in the accumulation of homocysteine in plasma and increase the risk of a premature cardiovascular disorder up to three times compared to the general population [39, 40].

According to the World Health Organization (WHO), cerebrovascular and neurodegenerative disorders affect one billion people around the world. A number of these disorders are characterized by the onset of dementia. Disability caused by dementia increases dramatically with aging, by affecting 9 per 1000 of the population aged 65-74 years to 83 per 1000 in the population over 85 years old [41]. Alzheimer's disease (AD) is the major cause of dementia in Western Countries, affecting 45-60% of the population, followed by vascular dementia and mixed dementia with prevalences of 30-40% and 10-20%, respectively [42, 43]. AD is a polygenic and complex disorder characterized by the accumulation of β-amyloid (Aβ) in senile plaques, neurofibrillary tangles, dendritic desarborization, and neuronal loss, which leads to memory deterioration, dementia, and functional decline [42-44].

The *PSEN1* and *PSEN2* genes, encoding presenilin1 and 2, are important determinants of the β-secretase activity responsible for proteolytic cleavage of the Aβ-precursor protein (APP). Mutations in the *PSEN1*, *PSEN2*, and *APP* confer phenotypes of amyloidogenic pathology and dementia [19, 42, 43, 45]. One of the most prevalent risk genes in AD is the *APOE*, especially in those individuals harboring the *APOE-ε4* allele [13, 19, 44, 46]. Interestingly, the allele *APOE-ε2*, which is associated with vascular risk, seems to be protective against dementia [13, 19, 44]. The *A2M* gene, encoding for the alpha-2-macroglobulin (a protease inhibitor), is also localized in amyloid plaques and interacts with Aβ and APOE. The polymorphism 2998G>A (rs669) in homozygosis increases the risk for the onset of AD by 4-fold when compared to the general population [13, 19, 45].

We characterized risk polymorphisms of genes related to obesity, cardiovascular disorders, and dementia in 4415 individuals from Spain. This population included a high rate of individuals diagnosed with vascular-related disorders (25%), and with dementia-related diseases (15%). The other 55% of the population was diagnosed with other pathologies that might be indirectly related to vascular or neurodegenerative disorders. We aimed to characterize which risk polymorphisms were predominant in our population and found that polymorphisms in *ACE*, *AGT(235)*, *IL6(573)*, *PSEN1*, and *APOE* (especially the *APOE-ε4* allele) were representative of our population as compared to reference data from Spanish and European individuals. The significantly high rate of the polymorphism +16G>T (rs165932) in the *PSEN1* gene, along with the high representation of the *APOE-ε4* allele, strongly suggests the presence of a significantly large group of individuals with potential AD disease onset in this population. The large number of individuals analyzed in this study may provide the main representative polymorphisms associated with risk for vascular disorders and dementia in the Spanish population.

2. MATERIALS AND METHODS

2.1. Subjects

Risk polymorphisms were analyzed in a population of 4415 individuals from Spain (72% from Galicia and Northern Spanish regions, and the remaining 18% from a widespread Spanish distribution) attending the outpatient clinic at the EuroEspes Biomedical Research Center from January 1995 to December 2015. The individuals were equally distributed in terms of gender, being 48.15% males and 51.85% females. Table **1** shows the distribution of the population according to age-range and diagnosis. The most representative age ranges were 30-60 years old (43%) and older than 60 (37%), whereas only 20% of individuals were younger than 30 years old. A representative number of those individuals (25%) were diagnosed with disorders associated with vascular damage or vascular risk, such as metabolic syndrome, brain trauma, cerebral stroke, epilepsy, cephalea. A total of 15% of individuals were diagnosed with dementia-related disorders, including AD, and vascular and mixed dementias; these last two being the most representative ones. Among neurodegenerative disorders, besides AD, approximately 4% of individuals were diagnosed with Parkinson's disease. Over 26% carried psychiatric disorders, including stress-anxiety and depression. The last 30% of patients were diagnosed with peripheral nervous system disorders and other pathologies (Table **1**).

2.2. Genotype Analysis

Blood was extracted in EDTA-coated tubes and DNA from peripheral blood was extracted using the Qiagen DNA Blood Minikit (Qiagen, Hilden, Germany). A total of 20 different genes were genotyped (Table **2**) by Real Time PCR (RT-PCR) using TaqMan assays designed for single nucleotide polymorphisms (SNPs). RT-PCRs were performed in Step One Plus Real Time PCR System (Life Technologies,

Waltham, Massachusetts, USA), and TaqMan® Open Array® DNA microchips in Quant Studio™ 12K Flex RT-PCR System (Life Technologies, Waltham, Massachusetts, USA). Open Array® genotyping analyses were performed using the Genotyper software provided by Thermo Fisher Scientific, Waltham, Massachusetts, USA.

Table 1. Description of the population characterized in this study: 4415 patients distributed throughout Spain (48.15% males and 51.85% females) who attended the outpatient clinic at the EuroEspes Biomedical Research Center (Spain) from January 1995 to December 2015.

Age Range	Age Rate (%)	Disease	Disease Rate (%)
0-15	7.18	Cephalea	9.33
16-30	13.16	Stress-Anxiety	18.10
30-60	43.10	Depression	8.18
>60	36.56	Dementia (AD,VD,MD)	14.75
—	—	Parkinson's	3.94
—	—	Cerebral Stroke	9.99
—	—	Epilepsy	4.01
—	—	PNS Disorders	1.18
—	—	Brain Trauma	0.45
—	—	Metabolic Syndrome	0.68
—	—	Others	29.40

AD: Alzheimer's Disease; VD: Vascular Dementia; MD: Mixed Dementia; PNS Disorders: Peripheral Nervous System Disorders.

2.3. Statistical Analysis

Deviation from Hardy-Weinberg equilibrium (HWE) was analyzed by a chi-square test (χ^2). Genotype distribution was considered in HWE when observed and theoretical distribution was similar with a $P>0.05$. The comparison of genotype frequencies in our population with others was analyzed using the Pearson's chi-square test, considering an equal genotype distribution when the two populations showed a similar distribution with a $P>0.05$.

2.4. Patient Consent

Written informed consent was obtained from all participants, or from a legal caregiver or representative on their behalf in case of incapability. This study and the consent procedures were approved by the institutional review board at 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 Journals Editors (ICMJE).

3. RESULTS

3.1. Polymorphisms in *ACE, AGT-235, IL6-573*, and *PSEN1* were Significantly More Represented in Our Population Compared to Representative Spanish and European Populations

Polymorphisms in a total of 20 selected genes associated with vascular risk and dementia-related neurodegeneration were analyzed. Gene polymorphisms were selected into panels according to i) previous research [47] and ii) genotyping in over 4000 patients. Genes were then classified into risk panels, defined as groups of genes and gene polymorphisms involved in the different steps (subpanels) which lead to the atherogenic process. In this regard, gene polymorphisms were classified into *Obesity*, *Vascular Risk*, and *Neurodegeneration* panels, and subpanels including *Lipid Metabolism, Endothelial Function, Hypertension, Immune Response*, and *Thrombosis* (Table **2**). Allele and genotype frequencies were calculated for each gene polymorphism. Risk alleles and their corresponding risk genotypes in homozygosis are in bold Table **2**.

A total of 8 out of the 20 polymorphism genotype distributions analyzed were not in Hardy-Weinberg equilibrium (HWE), these including *LPL* (χ^2=34.29; d.f.=2), *ACE* (χ^2=48.20; d.f.=2), *AGT174* (χ^2=9.37; d.f.=2), *AGT235* (χ^2=157.41; d.f.=2), *IL1B* (χ^2=4.88; d.f.=2), *IL6(174)* (χ^2=6.18; d.f.=2), *IL6(573)* (χ^2=76.31; d.f.=2), and *PSEN1* (χ^2=79.56; d.f.=2). Importantly, each polymorphism was analyzed in more than 900 patients (Table **2**), which rules out the possibility of a non-representative sample size. This finding suggested that those genes might be biased in our population, most probably due to the variety of vascular risk and dementia phenotypes in our patients.

Fig. (**1**) plots the risk allele frequencies of these polymorphisms outside HWE in our population (EuroEspes, black bars) with a differential distribution by comparison with other European populations, and especially with other Spanish groups (Mixed European: 1006 individuals; Spanish: 107 individuals; Italians: 107 individuals; British: 91 individuals; Finnish: 99 individuals. These data were extracted from "dbSNP" [48] and "1000 Genomes" [49] databases). In addition, polymorphisms in *APOC3* and *A2M*, which did not deviate from HWE in our population, also showed a differential risk allele frequency distribution when compared to another representative Spanish population (Fig. **1**). We used Pearson's chi-square test to assess whether the genotype distribution of the above mentioned genes in our population was significantly different from other representative European populations which do not deviate from HWE (Table **3**). The genes with a significant genotype distribution in our patients compared to representative Spanish populations were *ACE* (χ^2=9.49; P<0.01), *AGT-235* (χ^2=17.04; P<0.001), *IL6-573* (χ^2=10.99; P<0.005), and *PSEN1* (χ^2=13.59; P<0.0025) (Table **3**). Indeed, the genotype distribution of *IL6-573* and *PSEN1* in our population significantly differed from all the other European populations tested (P<0.05), whereas those distributions of *AGT-235* and *ACE* show similarities with a few other European populations (Table **3**).

Table 2. Allelic and genotype frequencies of different gene polymorphisms associated with obesity, vascular and cardiovascular risk, and neurodegeneration.

Panel [Subpanel]	Gene	OMIM	Locus	Polymorphism	Risk SNP	N	Allele Freq	Genotype Freq	H-W
-Obesity **-Vascular Risk** [Lipid Metabolism]	*APOB*	107730	2p24p23	rs693 [7545C>T]	7545T	931	C = 0.565 **T = 0.435**	CC = 0.319 CT = 0.492 **TT = 0.189**	Yes (P=0.65-0.7)
-Obesity **-Vascular Risk** [Lipid Metabolism]	*APOC3*	107720	11q23.3	rs5128 [3175G>C, S1/S2]	3175G	932	C = 0.906 G = 0.094	CC = 0.821 CG = 0.170 **GG = 0.009**	Yes (P=0.09-0.1)
-Obesity **-Vascular Risk** **-Neurodegeneration** [Lipid Metabolism]	*APOE*	107741	19q13.2	rs429358/rs7412 [112T>C/158T>C] E2, E3, E4	112T/158T (E2) 112C/158C (E4)	4377	ε3 = 0.815 **ε2 = 0.05** **ε4 = 0.135**	ε 3 ε 3 = 0.668 ε 2 ε 3 = 0.079 ε 2 ε 4 = 0.014 ε 3 ε 4 = 0.212 **ε 2 ε 2 = 0.002** **ε 4 ε 4 = 0.022**	Yes (P=0.35-0.4)
-Obesity **-Vascular Risk** [Lipid Metabolism]	*CETP*	118460	16q21	rs708272 [+279G>A, B1/B2]	+279G (B1)	2006	A = 0.377 **G = 0.623**	AA = 0.142 AG = 0.47 **GG = 0.388**	Yes (P=0.1-0.15)
-Obesity **-Vascular Risk** [Lipid Metabolism]	*LPL*	609708	8p22	rs328 [1421C>G, S474X]	1421G (Protective)	931	C = 0.851 **G = 0.149**	CC = 0.724 CG = 0.254 **GG = 0.022**	No (P<0.001)
-Vascular Risk **-Neurodegeneration** [Endothelial Function] [Hypertension]	*NOS3*	163729	7q36	rs1799983 [894G>T]	894T	2711	G = 0.621 **T = 0.379**	GG = 0.386 GT = 0.471 **TT = 0.144**	Yes (P=0.25-0.3)
-Vascular Risk **-Neurodegeneration** [Endothelial Function] [Hypertension]	*ACE*	106180	17q23.3	rs4332 [547C>T]	547T	954	C = 0.342 **T = 0.658**	CC = 0.117 CT = 0.450 **TT = 0.433**	No (P<0.001)
-Vascular Risk [Endothelial Function] [Hypertension]	*AGT-174*	1906150	1q42.2	rs4762 [9360G>A, T174M]	9360A	3453	G = 0.873 **A = 0.127**	GG = 0.762 GA = 0.222 **AA = 0.016**	No (P< 0.025)
-Vascular Risk [Endothelial Function] [Hypertension]	*AGT-235*	1906150	1q42.2	rs699 [9543A>G, M235T]	9543G	3453	A = 0.504 **G = 0.496**	AA = 0.254 AG = 0.500 **GG = 0.246**	No (P<0.001)
-Vascular Risk [Immune Response]	*IL1B*	147720	2q14	rs1143634 [3954C>T]	3954T	930	C = 0.795 **T = 0.205**	CC = 0.632 CT = 0.326 **TT = 0.042**	No (P<0.025)
-Vascular Risk [Immune Response]	*IL6 -174*	147620	7p21	rs1800795 [-174G>C]	-174C	930	G = 0.577 **C = 0.423**	GG = 0.333 GC = 0.489 **CC = 0.179**	No (P< 0.001)
-Vascular Risk [Immune Response]	*IL6 -573*	147620	7p21	rs1800796 [-573G>C]	-573C	930	G = 0.856 **C = 0.144**	GG = 0.733 GC = 0.247 **CC = 0.021**	No (P< 0.001)
-Vascular Risk [Immune Response]	*IL6R*	147880	1q21	rs8192284 [1510A>C]	1510C	930	A = 0.612 **C = 0.388**	AA = 0.375 AC = 0.475 **CC = 0.151**	Yes (P=0.85-0.9)
-Vascular Risk [Immune Response]	*TNFA*	191160	6p21.33	rs1800629 [-308G>A]	-308A	929	G = 0.854 **A = 0.146**	GG = 0.729 GA = 0.249 **AA = 0.021**	Yes (P=0.55-0.6)

(Table 2) contd….

Panel [Subpanel]	Gene	OMIM	Locus	Polymorphism	Risk SNP	N	Allele Freq	Genotype Freq	H-W
-Vascular Risk [Thrombosis]	*F2*	17693	11p11.2	rs1799963 [20210G>A]	20210A	931	G = 0.983 **A = 0.017**	GG = 0.966 GA = 0.033 **AA = 0.0002**	Yes (P=0.6-0.65)
-Vascular Risk [Thrombosis]	*F5*	227400	1q24.2	rs6025 [1691G>A]	1691A	932	G = 0.991 **A = 0.009**	GG = 0.982 GA = 0.018 **AA = 0.00008**	Yes (P=0.75-0.8)
-Vascular Risk [Thrombosis]	*MTHFR (A/C)*	607093	1p36.22	rs1801131 [1298A>C]	1298C	1004	A = 0.705 **C = 0.295**	AA = 0.497 AC = 0.416 **CC = 0.087**	Yes (P=0.55-0.6)
-Vascular Risk [Thrombosis]	*MTHFR (C/T)*	607093	1p36.22	rs1801133 [677C>T]	677T	2018	C = 0.624 **T = 0.376**	CC = 0.389 CT = 0.469 **TT = 0.141**	Yes (P=0.7-0.75)
-Neurodegeneration	*PSEN1*	104311	14q24.2	rs165932 [+16G>T]	+16G	2087	T = 0.429 **G = 0.571**	TT = 0.184 TG = 0.490 **GG = 0.326**	No (P< 0.001)
-Neurodegeneration	*A2M*	103950	12p13.31	rs669 [2998G>A]	2998G	2000	A = 0.694 **G = 0.306**	AA = 0.482 AG = 0.425 **GG = 0.094**	Yes (P=0.05-0.1)

A2M: alpha-2-macroglobulin; *ACE*: angiotensin I converting enzyme; *AGT*: angiotensinogen; *APOB*: apolipoprotein B; *APOC3*: apolipoprotein CIII; *APOE*: apolipoprotein E; *CETP*: cholesteryl ester transfer protein plasma; *F2*: coagulation factor II; *F5*: coagulation factor V; *IL1B*: Interleukin 1 beta; *IL6*: Interleukin 6; *IL6R*: Interleukin 6 receptor; *LPL*: lipoprotein lipase; *MTHFR*: methylenetetrahydrofolate reductase (NAD(P)H); *NOS3*: nitric oxide synthase 3; *PRNP*: prion protein; *PSEN1*: Presenilin 1; *TNFA*: tumor necrosis factor A. H-W: Hardy-Weinberg equilibrium. Significant deviation from H-W equilibrium (χ^2, P<0.05).

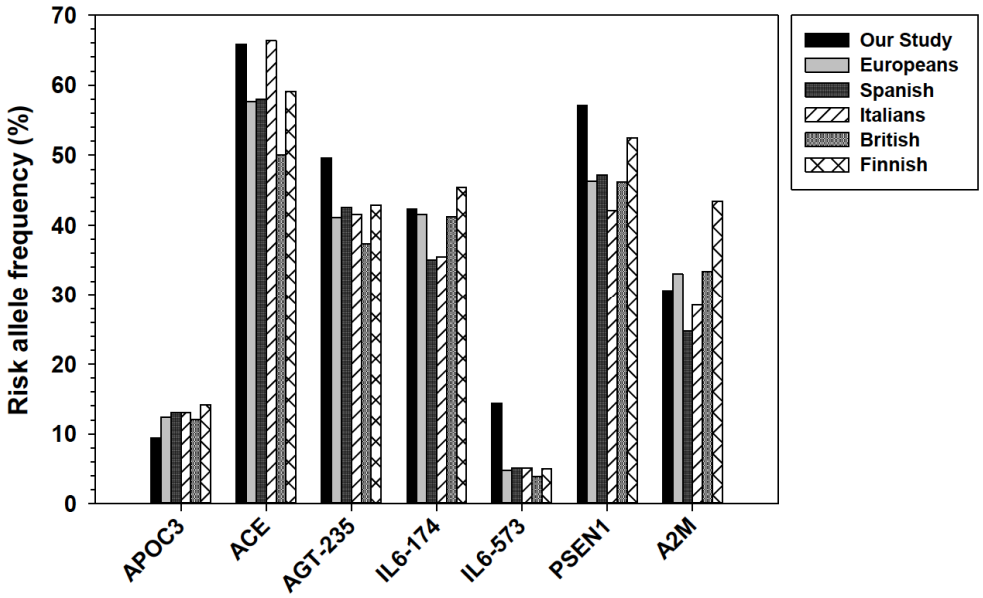

Fig. (1). Risk allele SNP frequencies with a differential distribution in our population compared to other European populations.
Populations: 1324 healthy individuals with a Northern and Western European ancestry extracted from the HapMap-CEU and Parc European studies (Ref.48); and 99, 91, 107, and 107 healthy individuals from Finland, Britain, Spain, and Italy, respectively, extracted from the 1000 Genomes database (Ref.49).

3.2. The *APOE-ε4* Allele is Highly Represented in Our Study Population

When comparing the distribution of all *APOE* alleles around the world, *APOE-ε3* is the most predominant with frequencies of 0.76 ± 0.09, followed by *APOE-ε4* (0.13 ± 0.07), and *APOE-ε2* (0.06 ± 0.02) [13]. It has been widely reported that the most prevalent risk factor for AD is an increased *APOE-ε4* distribution (0.36 ± 0.07) and decreased *APOE-ε2* (0.03 ± 0.02) [13, 19, 44, 46]. Allele distribution for *APOE* in our study population was apparently among the usual in general European populations [13]: *APOE-ε3*=0.815, *APOE-ε4*=0.135, and

Table 3. Comparative distribution of genotype frequencies of gene polymorphisms associated with obesity, vascular/cardiovascular risk, and neurodegeneration in our study and other European populations [48, 49].

—	—	Genotype Frequencies						
Gene	**Genotypes**	**Our Study**	**N-W Europeans**	**Western Europeans**	**Finnish**	**British**	**Spanish**	**Italian**
A2M	AA AG GG	0.482 0.425 0.093	0.465 0.446 0.089 **$\chi2 = 0.07$ P>0.95**	0.625 0.250 0.125 **$\chi2 = 7.13$ P<0.05**	0.333 0.465 0.202 **$\chi2 = 18.31$ P<0.001**	0.428 0.495 0.077 **$\chi2 = 0.97$ P>0.65**	0.551 0.402 0.047 **$\chi2 = 3.46$ P>0.2**	0.504 0.421 0.075 **$\chi2 = 0.44$ P=0.8**
ACE	CC CT TT	0.117 0.450 0.433	 -- --	0.149 0.474 0.376 **$\chi2 = 5.78$ P>0.05**	0.152 0.515 0.333 **$\chi2 = 11.22$ P<0.005**	0.263 0.473 0.264 **$\chi2 = 16.43$ P<0.001**	0.178 0.486 0.336 **$\chi2 = 9.49$ P<0.01**	0.121 0.43 0.449 **$\chi2 = 3.29$ P>0.15**
AGT-174	GG GA AA	0.762 0.222 0.016	0.791 0.200 0.009 **$\chi2 = 1.97$ P>0.4**	0.783 0.217 0.000 **$\chi2 = 1.08$ P>0.55**	0.627 0.343 0.030 **$\chi2 = 10.59$ P=0.005**	0.791 0.209 0.000 **$\chi2 = 2.07$ P>0.35**	0.803 0.178 0.019 **$\chi2 = 0.75$ P>0.65**	0.757 0.215 0.028 **$\chi2 = 0.19$ P>0.9**
AGT-235	AA AG GG	0.254 0.500 0.246	0.371 0.434 0.195 **$\chi2 = 39.89$ P<0.001**	0.218 0.652 0.130 **$\chi2 = 1.21$ P>0.5**	0.303 0.535 0.162 **$\chi2 = 6.28$ P<0.05**	0.384 0.484 0.132 **$\chi2 = 18.5$ P<0.001**	0.355 0.439 0.206 **$\chi2 = 17.04$ P<0.001**	0.365 0.439 0.196 **$\chi2 = 18.55$ P<0.001**
APOC3	CC CG GG	0.821 0.170 0.009	1.000 0.000 0.000 **$\chi^2 = 46.00$ P<0.001**	0.841 0.159 0.000 **$\chi2 = 0.63$ P>0.7**	0.727 0.263 0.010 **$\chi2 = 6.43$ P<0.05**	0.770 0.220 0.010 **$\chi2 = 2.03$ P>0.35**	0.766 0.206 0.028 **$\chi2 = 3.05$ P>0.2**	0.757 0.224 0.019 **$\chi2 = 3.00$ P>0.2**
IL1B	CC CT TT	0.632 0.326 0.042	0.159 0.310 0.531 **$\chi2 = 359.5$ P<0.001**	0.583 0.250 0.167 **$\chi2 = 10.55$ P<0.01**	0.566 0.394 0.040 **$\chi2 = 3.61$ P>0.15**	0.450 0.473 0.077 **$\chi2 = 13.41$ P<0.0025**	0.617 0.374 0.009 **$\chi2 = 5.62$ P>0.05**	0.533 0.402 0.065 **$\chi2 = 5.15$ P>0.05**
IL6-174	GG GC CC	0.333 0.489 0.179	0.247 0.434 0.319 **$\chi2 = 17.99$ P<0.001**	0.208 0.542 0.250 **$\chi2 = 4.24$ P>0.1**	0.313 0.465 0.222 **$\chi2 = 0.7$ P>0.65**	0.373 0.429 0.198 **$\chi2 = 0.17$ P>0.95**	0.411 0.477 0.112 **$\chi2 = 4.9$ P>0.1**	0.420 0.449 0.131 **$\chi2 = 3.52$ P>0.15**
IL6-573	GG GC CC	0.733 0.247 0.021	0.914 0.086 0.000 **$\chi2 = 26.62$ P<0.001**	0.990 0.010 0.000 **$\chi2 = 13.01$ P<0.0025**	0.899 0.101 0.000 **$\chi2 = 10.41$ P<0.01**	0.923 0.077 0.000 **$\chi2 = 12.41$ P<0.025**	0.897 0.103 0.000 **$\chi2 = 10.99$ P<0.005**	0.897 0.103 0.000 **$\chi2 = 10.99$ P<0.005**
LPL	CC CG GG	0.724 0.254 0.022	0.766 0.217 0.017 **$\chi2 = 4.02$ P>0.1**	0.545 0.364 0.091 **$\chi2 = 9.87$ P<0.01**	0.788 0.192 0.020 **$\chi2 = 1.67$ P>0.4**	0.791 0.187 0.022 **$\chi2 = 1.43$ P>0.45**	0.701 0.271 0.028 **$\chi2 = 2.97$ P>0.2**	0.776 0.196 0.028 **$\chi2 = 0.85$ P>0.65**
PSEN1	TT TG GG	0.184 0.490 0.326	0.319 0.504 0.177 **$\chi^2 = 54.47$ P<0.001**	0.435 0.391 0.174 **$\chi^2 = 34.27$ P<0.001**	0.222 0.505 0.273 **$\chi^2 = 6.05$ P<0.05**	0.285 0.506 0.209 **$\chi^2 = 16.06$ P<0.001**	0.261 0.533 0.206 **$\chi^2 = 13.59$ P<0.0025**	0.336 0.486 0.178 **$\chi^2 = 33.73$ P<0.001**

A2M: alpha-2-macroglobulin; *ACE*: angiotensin I converting enzyme; *AGT*: angiotensinogen; *APOC3*: apolipoprotein CIII; *CETP*: cholesteryl ester transfer protein plasma; *IL1B*: Interleukin 1 beta; *IL6*: Interleukin 6; *LPL*: lipoprotein lipase; *PSEN1*: Presenilin 1.

Populations: 1301 healthy individuals with a Northern and Western European ancestry extracted from the HapMap-CEU study (N-W Europeans) (Ref.48); 23 healthy individuals from France and Utah residents with European ancestry (Western Europeans) (Ref.48); and 99, 91, 107, and 107 healthy individuals from Finland, Britain, Spain, and Italy, respectively, extracted from the 1000 Genomes database (Ref.49).

APOE-ε2=0.050 (Table **2**). Indeed, despite the representative number of individuals with vascular risk and dementia in our group, distribution of *APOE-ε2* and *APOE-ε4* appeared within the range of those of other European and Worldwide populations [50-67] (Fig. **2**). It is widely documented that the *APOE-ε4* allele rate is lower in Spanish and South European populations [50, 64-67], but the levels of *APOE-ε4* in our study population are rather higher than those of South Europeans, including Spanish individuals (Fig. **2**), which, once again, might be related to the rate of dementia and atherosclerosis risk in our group. *APOE-ε2* distribution is also slightly lower in our population compared to all the other groups, although still far from those with AD (grey bars, Fig. **2**).

In order to establish the influence of *APOE-ε4* and *APOE-ε2* distribution in our population, we performed a comparative analysis of the *APOE* genotype variants with other representative Spanish populations [51, 52, 54, 56, 58, 61, 63-67] (Fig. **3A**). *APOE* genotypes containing the alleles *ε2* and *ε3* (*ε2/ε2*, *ε2/ε3*, and *ε3/ε3*) did not display large differences between our population and average Spanish groups. However, genotypes containing the *ε4* allele were much more evident in our population, especially the homozygous *ε4/ε4*, with a 2.5-fold increase, followed by the heterozygous *ε2/ε4*, with a near 2-fold increase, and *ε3/ε4* showing an approximately 1.5-fold increase (Fig. **3B**). Pearson's chi-square test provided evidence of a significantly different distribution of the *APOE* genotypes in our population as compared to five separate representative Spanish populations ($P<0.01$-0.001) (Table **4**), which strongly suggests the high influence of the *APOE-ε4* allele in our population. Those particularly higher levels of *APOE-ε4* and *PSEN1* may be a sign of a representative number of individuals with AD in our group. Nevertheless, the *APOE-ε4* rate in our group still far differs from Spanish individuals with AD ($P<0.001$) Table **4**. Although *APOE* genotypes involving the *ε4* allele (*ε4/ε4*, *ε2/ε4*, and *ε3/ε4*) are significantly higher in our population compared to other Spanish groups, it is nevertheless sited within the range values of average European and Worldwide populations, unlike the *ε2/ε2* genotype (Fig. **4**).

4. DISCUSSION

This study evaluates the impact of 20 gene polymorphisms associated with vascular risk and dementia (Table **2**) in a broad population of 4415 individuals from Spain carrying a variety of disorders involving metabolic syndrome, vascular damage, and impaired cognitive decline leading to dementia, among others Table **1**. A total of approximately 40% of patients were diagnosed with disorders involving, directly or indirectly, vascular damage or cognitive decline leading to dementia. A significant part of those patients with dementia have a cerebrovascular component. Some of the remaining 60% of pathologies (psychiatric disorders, peripheral nervous system (PNS) impairment, or others) might also lead to vascular damage or dementia, depending on the disease progression or genetic predisposition of the individuals.

Genotype distributions in 8 out of the 20 polymorphisms analyzed (*LPL, ACE, AGT-174, AGT-235, IL1B, IL6-174, IL6-573, PSEN1*) deviate from HWE Table **2**. In this case, the number of individuals analyzed was large enough to be representative. Therefore, those gene polymorphisms were good candidates to distinguish our biased group from average populations. However, when the genotype distribution of these genes in our group was compared with other representative Spanish or European populations, we found that only the polymorphisms *ACE* (547C>T, rs4332), *AGT* (9543A>G, M235T, rs699), *IL6* (573G>C, rs1800796), and *PSEN1* (16G>T, rs165932) were representative in our population (Fig. **1**; Table **3**).

Hypertension is one of the leading risks (50-70%) for cardiovascular disorders and cerebral stroke, which are considered to be among the top three causes of death in the US and Europe [6, 7]. Polymorphisms in genes involved in blood pressure regulation are markedly represented in our population. Genes encoding for angiotensin I-converting enzyme (*ACE*) and the angiotensinogen (*AGT*) are involved in the renin-angiotensin system pathway that regulates blood pressure. Polymorphisms in those genes have been reported to be associated with hypertension [13, 24-27]. Polymorphisms in inflammation-related genes such as *IL6* are also involved in hypertension and atherosclerosis [11, 13, 28, 31, 33], although the physiological mechanisms are not well characterized.

Apparently, hypertension-related polymorphisms, rather than lipid metabolism-related variations, are the major genetic cause for vascular disorders in our population. Indeed, correlation studies performed in Spanish populations demonstrate that regions with a higher total fat consumption presented a lower coronary-leading mortality, and vice versa [68, 69]. This finding suggests (i) the strong influence of genetic predisposition over environmental factors; (ii) the lower importance of variations in genes involved in lipid metabolism (*APOB, APOC3, CETP*) compared to those involving blood pressure regulation in Spanish populations.

The only gene involved in lipid metabolism with a representative influence in our population was *APOE*, and particularly, the *APOE-ε4* allele (Figs. **2-4**, Table **4**). It is widely reported that the increased risk of developing an ischemic cardiopathy, which is directly proportional to the *APOE-ε4* allele frequency, follows a north-south gradient throughout Europe, being more frequent in northern than southern countries [50, 64-67, 70, 71]. Although the *APOE-ε4* allele frequency in our population fitted within the range of European individuals, it was nevertheless higher than the average Spanish and South European populations (Fig. **2**). In addition, all genotype variants involving *APOE-ε4* were predominant in our population compared to other Spanish groups (Fig. **3**, Table **4**), but not to other Europeans or Worldwide populations (Fig. **4**).

APOE-ε4, and especially the *APOE-ε4/ε4* genotype, is one of the most prevalent risk factors associated with neurological hallmarks of AD [13, 19, 44, 46]. It has been reported that *APOE-ε4* may influence AD by interacting with APP metabolism and Aβ accumulation, enhancing the hyperphosphorylation of tau protein, and starting a chain reaction involving oxidative processes, modification of the neuroimmunotrophic activity, altering lipid metabolism and transport, and membrane biosynthesis in sprouting and synaptic remodeling, and inducing apoptosis [13, 19, 44, 72-74].

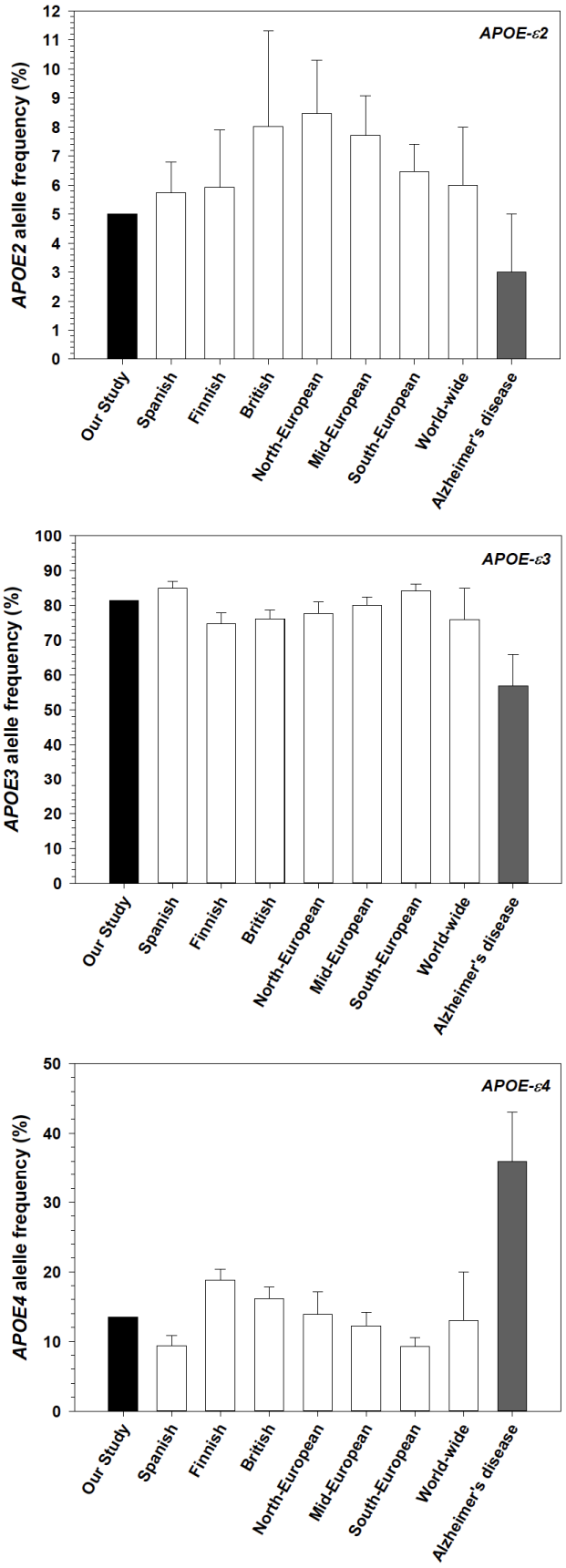

Fig. (2). Distribution of the *APOE* alleles in our study compared to other Spanish, European, and Worldwide populations, including healthy individuals, and patients with Alzheimer's disease [50-67].

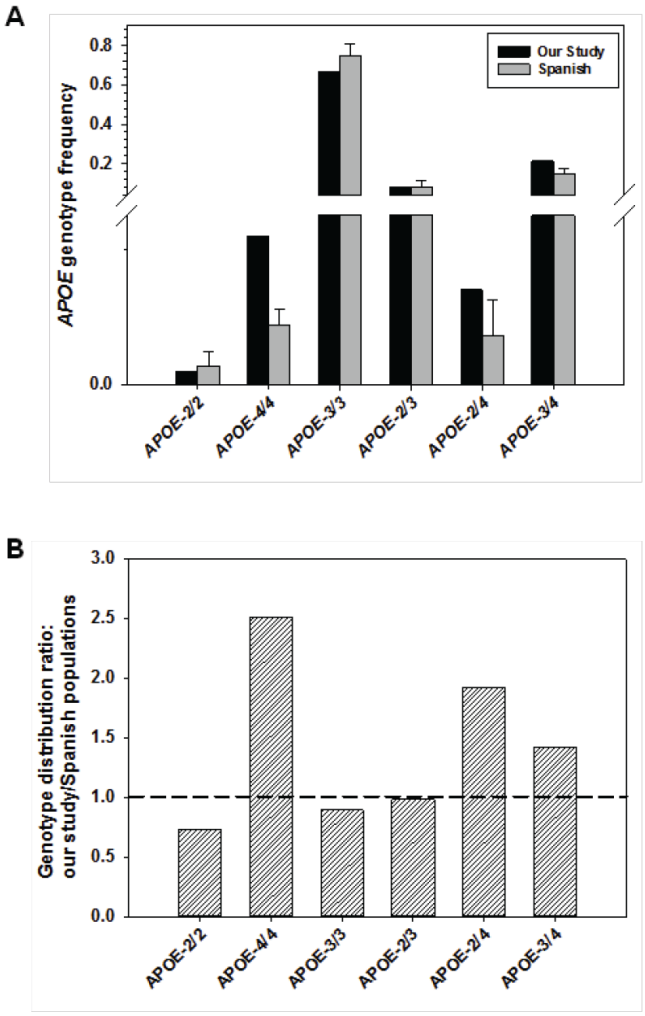

Fig. (3). Distribution of *APOE* genotypes in our study and in five other representative Spanish populations [51, 52, 54, 56, 58, 61, 63-67] (A). Genotype distribution ratio between our population (bars) and the other five Spanish populations (dashed line) (B). Spanish groups description: control (healthy) individuals from a broad distribution of Spanish regions (n=1286), and other groups from specific regions, such as Asturias (n=250), Barcelona (n=478), and Navarra (n=188).

However, other different reasons may also explain the high rate of *APOE-ε4*, and the *APOE-ε4/ε4* genotype in our population. The *APOE-ε4* allele is highly associated with an aberrant increase in LDL-family lipoproteins and cholesterol, which promotes the risk of developing atherosclerosis and vascular disorders [13-20], which may be explained by the significant rate of disorders with a vascular component represented in our population. Some of the individuals belonging to the 15% carrying dementia-related disorders were diagnosed with AD, although a high proportion of them had a vascular component rather than neurodegenerative. In addition, *APOE*-related pathogenic mechanisms are also associated with brain aging [75], and over 35% of individuals in our population were older than 60.

The presence of *APOE-ε4* is a hallmark but not enough to cause AD. Nevertheless, besides the prevalence of *APOE-ε4*, there are also other good reasons to suggest that our population may have a higher rate of individuals with a potential

Table 4. Genotype frequencies of *APOE* polymorphisms in our population and different Spanish populations of healthy individuals (Control) or individuals with Alzheimer's disease (AD).

—	—	Genotype Frequencies								—
Gene	**Genotypes**	**Our Study**	**Spanish Control**	**Spanish Control**	**Spanish Control**	**Spanish Control**	**Spanish Control**	**Spanish AD**	**Spanish AD**	**References**
APOE	*ε-2/2*	0.002	0.005	0.002	0.005	0.002	0.000	0.000	0.001	[51, 52, 54, 56, 58, 61] [63-67]
	ε-4/4	0.022	0.005	0.008	0.010	0.011	0.010	0.080	0.092	
	ε-3/3	0.668	0.780	0.732	0.672	0.731	0.830	0.580	0.531	
	ε-2/3	0.079	0.100	0.089	0.116	0.070	0.027	0.020	0.051	
	ε-2/4	0.014	0.000	0.009	0.014	0.009	0.005	0.000	0.020	
	ε-3/4	0.212	0.120	0.155	0.164	0.178	0.128	0.320	0.296	
—	—	—	$\chi^2 = 23.37$ P<0.001	$\chi^2 = 34.43$ P<0.001	$\chi^2 = 16.71$ P<0.01	$\chi^2 = 12.23$ P<0.01	$\chi^2 = 22.44$ P<0.001	$\chi^2 = 27.99$ P<0.001	$\chi^2 = 29.43$ P<0.001	—

Spanish groups description: control (healthy) individuals from a broad distribution of Spanish regions (n=1286), and other groups from specific regions, such as Asturias (n=250), Barcelona (n=478), and Navarra (n=188). Table also includes two Spanish groups with individuals diagnosed with Alzheimer Disease from Navarra (n=98) and Asturias (n=120).

onset of AD than previously expected: (i) Polymorphism +16G>T (rs165932) in the *PSEN1* gene is also prevalent in our population compared to other representative Spanish and European populations (Table **3**). This polymorphism is a hallmark of AD, since it affects β-secretase activity leading to accumulation of Aβ. [19, 42, 43, 45]. (ii) Contrary to *APOE-ε4*, carriers of *APOE-ε2* may be protected against dementia, and in fact, *APOE-ε2* allele distribution is usually lower in individuals with AD [13, 19, 44]. Our population displays a higher rate of *APOE-ε4* and lower *APOE-ε2* than other Spanish and South European populations (Figs. **2**,**3**). (iii) Several reports associate metabolic syndrome and diabetes as promoters of future AD onset [76-78], which would suggest a prognosis for a potential number of individuals in our population. However, some of those reports define as AD cases of dementia associated with a vascular component turning into neurodegeneration, which is not entirely correct.

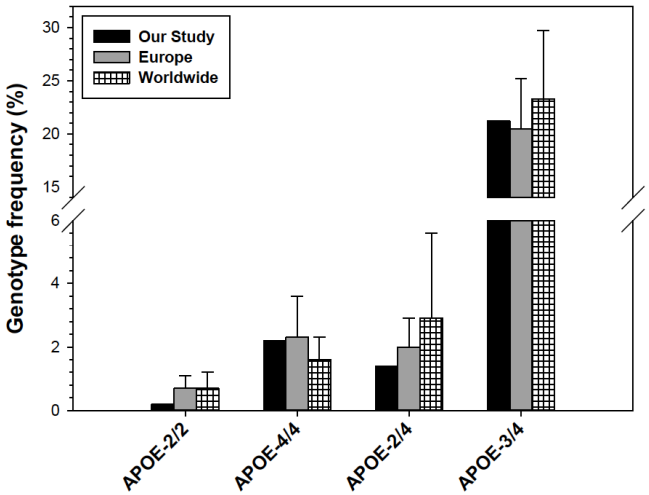

Fig. (4). Genotype frequencies of the *APOE-ε2/2* (A), *APOE-ε4/4* (B), *APOE-ε2/4* (C), and *APOE-ε3/4* (D) in our population, compared to representative European and Worldwide populations [13, 50, 51, 61]. Error bars correspond to STD.

CONCLUSION

The objective of this study was to characterize the influence of gene polymorphisms related to vascular risk and dementia on a broad population of Spanish individuals diagnosed with a variety of disorders concerning impaired lipid metabolism, hypertension, atherosclerosis, and dementia, among others. Polymorphisms in *ACE*, *AGT(235)*, *IL6(573)*, *PSEN1*, and *APOE* (especially the *APOE-ε4* allele) were representative of our study population as compared to reference data of Spanish and European individuals. Results obtained in this work may yield the following conclusions: (i) the large number of individuals analyzed in this study might provide a hallmark of cardiovascular and dementia risk polymorphisms in the Spanish population; (ii) polymorphisms involving hypercholesterolemia are more pronounced in our population, rather than those related to lipid metabolism; (iii) the prevalence of *APOE-ε4* and +16G>T (rs165932) variation in the *PSEN1* gene suggests a higher potential rate of AD onset in our population. Thus, AD diagnosis might be underestimated in our population, or not yet diagnosed since the classic symptoms had not yet shown up, although they might already be genetically imprinted.

Identification of risk polymorphisms provides information about the susceptibility of an individual for the onset of certain diseases, compared to the average population. A proper risk assessment requires the analysis of variations in multiple potential interacting genes at individual, family, and ethnical levels. Identification and proper analysis of risk polymorphisms will ensure more reliable, accurate, and affordable treatments.

CONFLICT OF INTEREST

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