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Genomics and Pharmacogenomics of Cerebrovascular Disorders

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ABSTRACT

Cerebrovascular accidents occur by the interaction of multiple environmental factors and mutations in different genes. These genetic polymorphisms determine susceptibility or resistance to disease and response to treatment. Due to the interaction between genes and environment, diseases are preventable through action on the environmental factors with an adequate prevention plan.

Genetic testing for cerebrovascular diseases establishes the susceptibility, risk or probability of an individual suffering the disease. For this reason, the results of the tests merely indicate that a person may have a greater probability, risk or susceptibility to suffering the disease than the population at large, but it does not mean that the person will necessarily suffer this disease, as this risk is influenced by other variables, such as external conditioning factors.

Genetic panels for cerebrovascular risk study different genetic polymorphisms or variations in the DNA sequence, which are involved in the development, prognosis and evolution of these pathologies, and represent a key tool in medical practice.

In cerebrovascular treatments, variability in efficacy and serious adverse effects continue to plague therapy. There are many sources of variability in response to drug therapy, such as noncompliance and unrecognized drug interactions, but translating pharmacogenomic discoveries to individual patients and populations is a challenge for genomic and personalized medicine.

We review different findings in the field of predictive genomics and pharmacogenomics to improve prevention, diagnosis and treatments.This review describes environmental and genetic factors involved in the disease as well as major pharmacogenes coding for enzymes, receptors and transporters involved in ADME processes, such as absorption, distribution, metabolism and excretion of drugs, used in cerebrovascular therapy: antithrombotic agents, antihypertensives, antiarrhythmics, beta-blockers, and lipid modifying agents.

Keywords

Antiarrhythmics, Antihypertensives, Antithrombotic agents, Atherosclerosis, Beta-blockers, Calcium channel blockers, Genetic risk, Genomics, Lipid modifying agents, Pharmacogenetics, Stroke, Vascular risk factors

INTRODUCTION

Cerebrovascular accidents occur by the interaction of multiple environmental factors and mutations in different genes. These genetic polymorphisms determine susceptibility or resistance to disease and response to treatment. Due to the interaction between genes and environment, some diseases are preventable through action on the environmental factors with a personalized prevention plan.

17.5 million people died from cardiovascular diseases in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to cerebrovascular accident or stroke [1]. Cerebrovascular disorders and stroke are the fourth leading cause of death behind diseases of the hearth, cancer, and chronic lower respiratory disease, in the US and in Europe with around 200 cases per 100,000 inhabitants per year [2] and almost six million victims every year, according to the American Heart Association [3].

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Prevalence of stroke was 33 million, with 16.9 million people having a first stroke. 5.2 million (31%) first strokes were in those <65 years of age [4]. There were an estimated 11.6 million events of incident ischemic stroke and 5.3 million events of incident hemorrhagic stroke, 63% and 80%, respectively [5]. Stroke was the second-leading global cause of death behind ischemic heart disease, accounting for 11.13% of total deaths worldwide [6]. 2.8 million individuals died of ischemic stroke and 3.0 million of hemorrhagic stroke (57% and 84%, respectively) [5].

Genetic susceptibility is the probability of an individual of developing a particular disease as a result of their genetic profile and external conditions. Genetic testing of cerebrovascular disorders determines susceptibility of an individual developing the disease. Therefore, the test result indicates only that the patient may be more likely than most people to suffer the disease, but does not mean that it will be suffered, as this risk is conditioned by other variables. Design of a personalized health strategy based on a particular genetic profile will adapt the external conditions such as diet and lifestyle (exercise, alcohol consumption, smoking, etc.), plus drug treatment and use of nutraceuticals or functional foods, to intervene in the individual's susceptibility to develop the disease.

Cerebrovascular diseases refer to disorders of the brain blood vessels that affect the blood supply to the brain. According to its etiology they are usually classified into ischemic or hemorrhagic strokes, and depending of the origin of the disorder, susceptibility genes involved in the increase of risk will be different.

Ischemic Stroke

Ischemic stroke (cerebral infarction) is due to occlusion of one of the arteries supplying blood to the brain, usually by atherosclerosis. We can distinguish: a) transient ischemic attack (TIA), episode of a focal deficit of cerebral circulation, with sudden onset, with alterations that usually last about 2-10 minutes but may persist for up to 24 hours, b) reversible ischemic neurologic deficit (RIND), the duration of the deficit is more than 24 hours, but the clinical signs and symptoms disappear totally during the three weeks following the episode, c) cerebral infarction, as the result of the lack of blood supply to a brain territory, a neurological deficit of more than 24 hours duration is presented, the infarction may be silent (SCI), but usually gives neurological manifestations according to the affected territory, d) atherothrombotic cerebral infarction (ACI), injury of the vessel wall that causes a stenosis or occlusion of the arterial lumen and an injury occurs within its territory irrigation that can be total or partial, depending on the possible compensation of collateral circulation.

Hemorrhagic Stroke

Hemorrhagic stroke (cerebral hemorrhage) is due to rupture of a cerebral blood vessel due to a hypertensive peak or a congenital aneurysm. Types of brain hemorrhages: a) cardioembolic cerebral infarction (CI), heart valve injury, infarction and/or heart rhythm disorders give rise to blood clots that reach the brain arteries, b) hemorrhagic cerebral infarction, it occurs on ischemic hemorrhagic injury background altering the blood brain barrier in an area of reperfusion, usually after lysis of the thrombus, c) lacunar infarctionis a small, less than 15 mm, resulting from occlusion of one of the penetrating arteries that provides blood to the brain's deep structures, d) intracerebral hemorrhage is a collection of blood within the brain parenchyma due to rupture of a cerebral vessel.

Atherosclerosis

Disabling and often fatal complications of cerebrovascular disease appear in the last stage of life. However, atherosclerosis, the main pathological process leading to cerebrovascular disease, begins in youth or adulthood, remaining asymptomatic for 20 or 30 years until the onset of the disease [7,8].

Atherosclerosis is a specific form of arteriosclerosis that affect arterial blood vessels due to the invasion and accumulation of white blood cells (monocyte-derived macrophages and T cells) resulting from interaction between modified lipoproteins and the normal cellular elements of the arterial wall. This inflammatory process leads to complex lesions, or atheroma plaques, that protrude into the arterial lumen. Plaque rupture and thrombosis results in the acute clinical complications of myocardial infarction and stroke [9,10].

Lipid Metabolism

In the late 1970s, different authors postulated the importance of oxidation of LDL cholesterol as an initiating event of the atherogenic process and the accumulation of modified LDL-cholesterol within macrophages resulting in so-called foam cells [11-13].

Description of the molecular mechanisms underlying cholesterol biosynthesis and regulation of serum cholesterol levels by Goldstein and Brown [14], highlighted the importance of different proteins involved in lipid metabolism as ApoB, ApoC-III, and ApoE, as well as the LDL receptor, which is essential in recognizing ApoB in LDL, as well as LPL that hydrolyzes the triglyceride in plasma chylomicrons and VLDL, releasing free fatty acids for uptake by peripheral tissues [15].

Endothelial Function and Hypertension

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. 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. Contribution of endothelial nitric oxide synthase (eNOS) in LDL-cholesterol oxidation, foam cell formation and endothelial equilibrium have been described by Knowles et al. [16], who suggest that deletion of the *eNOS* gene in the background of ApoE deficiency results in hypertension and increased atherosclerosis.

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, both play crucial roles in endothelial function and in profusion of the atherosclerotic plaque [17-20].

Immune Response and Inflammation

Oxidized LDL particles are cytotoxic and proinflammatory, and monocyte/macrophage recruitment is regulated by cell adhesion molecules and cytokines expressed by endothelial cells [21].

Circulating markers of inflammation are associated with 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 [22-30].

Plaque Rupture and Thrombosis

Thickening of the plaque eventually leads to a break in the same and subsequent atherothrombotic process. Extracellular accumulation of cholesterol, the release of metalloproteinases by macrophages and initiation of the coagulation cascade favor the formation of clots, emboli and thrombi ultimately lead to stroke [31,32]. 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 [33-37].

Cerebrovascular Risk Factors

More than 300 risk factors have been associated with cardiovascular disease and stroke. 80% of all strokes are preventable and it starts with managing key risk factors (**Table 1**). Risk factors for stroke should meet three basic criteria: i) high prevalence in different populations,ii) clear cause and effect relationship, and, iii) treatment and control reduce the incidence of stroke.

Hypertension

High blood pressure (hypertension) is defined as a systolic blood pressure (SBP) above 140 mmHg and/or a diastolic blood pressure (DBP) above 90 mmHg. In most developed countries, up to 30% of people aged up to 25 years suffer from high blood pressure [38].

In 2000, it was estimated that 972 million adults worldwide had hypertension [39] and it is know that high blood pressure (HBP) is a major risk for cardiovascular disease and stroke. Approximately 77% of people who have a first stroke have BP ≥140/90 mmHg [40].

Numerous risk factors and markers for development of hypertension have been identified, including age, ethnicity, family history of hypertension and genetic factors, lower education and socioeconomic status, greater weight, lower PA, tobacco use, psychosocial stressors, sleep apnea, and dietary factors (including dietary fats, higher sodium intake, lower potassium intake, and excessive alcohol intake) [38].

Dyslipidemia

Dyslipidemia is an abnormal amount of lipids in the blood. In developed countries, most dyslipidemias are hyperlipidemias, and usually due to high total cholesterol and/or low-density lipoprotein-cholesterol (LDL-cholesterol) levels [41]. Dyslipidemias cover a broad spectrum of lipid abnormalities, some of which are of great importance in cerebrovascular disease prevention. Dyslipidemias may be related to other diseases (secondary dyslipidemias) or to the interaction between genetic predisposition and environmental factors.

Hypercholesterolemia is defined as total cholesterol above 220 mg/dl and/or LDL-cholesterol above 160 mg/dl. Elevated levels of cholesterol in the blood may be a consequence of diet, obesity, inherited diseases, as familial hypercholesterolemiadue to *LDLR* mutations, or diabetes, and is considered one of the major risk factors leading to heart disease, heart attack and stroke [42]. A meta-analysis of 17 prospective trials found hypertriglyceridemia to be an independent risk factor for cardiovascular disease [43]. Other types of dyslipidemias appear to predispose to premature cardiovascular disease. A particular pattern, termed the atherogenic lipid triad [42] or atherogenic lipid phenotype [43], is more common than others, and consists of the co-existence of increased very low density lipoprotein (VLDL) remnants manifested as mildly elevated triglycerides (TG), increased small dense low-density lipoprotein (LDL) particles, and reduced high-density lipoprotein-cholesterol (HDL-C) levels.

Smoking

In 2010, tobacco smoking was the second-leading risk factor of death in USA, after dietary risks [44]. Annually from 2005 to 2009, smoking was responsible for a half million premature deaths in among those ≥ 35 years of age.

Furthermore, almost one third of deaths of coronary heart disease are attributable to smoking and secondhand smoke exposure [45].

Worldwide, tobacco smoking (including secondhand smoke) was one of the top three leading risk factors for disease and contributed to an estimated 6.2 million deaths in 2010 [46]. On average, male smokers die 13.2 years earlier than male nonsmokers, and female smokers die 14.5 years earlier than female nonsmokers [47].

Table 1. Management of cerebrovascular risk factors

Risk Factor	Status	Clinical feature	Pharmacological intervention	Behavioral intervention	Genetic markers	Pharmacogenetics
High blood pressure	Modifiable	Hypertension (SBP>140 mmHg, DBP>90 mmHg)	Antihypertensive drugs, Agents acting on the renin-angiotensin system, Diuretics	Low salt diet, weight control, exercise	*ACE, AGT, NOS3*	*ABCB1, CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP3A5, NAT2, SLCO1B1, UGT1A1*
Abnormal blood lipids	Modifiable	Hypercholesterolemia (Total-chol>220 mg/dl, LDL-chol>160 mg/dl), Hypertriglyceridemia (Triglyc>150 mg/dl)	Lipid modifying agents (statins, fibrates)	Low saturated fat diet	*APOA2, APOA5, APOB, APOC3, APOE, CETP, FABP2, LPL*	*ABCB1, CYP1A2, CYP2C9, CYP2D6, CYP3A4, CYP3A5, SLCO1B1, UGT1A1*
Smoking	Modifiable	Tabaquism		Smoking cessation	*ANKK1, CHRNA4, CHRNA5, CHRNB2, DDC, DRD1, DRD2, DRD3, NRXN1, NTRK2, SNCA*	*CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2C9, CYP2D6, CYP2E1, CYP3A4, CYP3A5, UGT1A1*
Physical inactivity	Modifiable	Physical inactivity		Exercise	*ADRB2, APOC3, LIPC, TCF7L2*	
Obesity	Modifiable	Obesity (BMI>30 kg/m^2)	Antiobesity drugs, Nutraceutical intervention	Low calorie diet, weight control, exercise	*ADRB2, ADRB3, ANKK1, CLOCK, FTO, LEP, LEPR, MC4R, NPY, PLIN, TAS1R2, TNF, UCP2*	*CYP1A2, CYP2C19, CYP2C9, CYP3A4, CYP3A5*
Unhealthy diet	Modifiable	Deficiency syndrome, low fruit and vegetables intake, high saturated fat intake	Nutraceutical supplementation	Equilibrated diet (increase fruits and vegetables in diet)		
High blood	Modifiable	Diabetes	Blood glucose lowering	Low	*ADRB2,*	*CYP1A2, CYP2C19,*

glucose levels		mellitus,Hyperglycemia	drugs	carbohydrate diet	*APOC3, FABP2, FTO, KCNJ11, SLC2A2, SLC30A8, TCF7L2, TNF*	*CYP2C9, CYP2D6, CYP3A4, CYP3A5, G6PD, NAT2, SLCO1B1, UGT1A1*
Alcohol	Modifiable	Alcoholism		Alcohol cessation	*ADH, ALDH1, ALDH2*	*CYP2E1*
Mental disorders	Modifiable	Depression	Antidepressants	Psychotherapy		*ABCB1, CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP3A5, UGT1A1*
Psychosocial stress	Modifiable	Anxiety and chronic life stress	Anxiolytics	Psychotherapy	*DBH, FKBP5, SERT*	*CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP3A5, UGT1A1*
Drug misuse	Modifiable	Oral contraceptives and hormone replacement therapies	Oral contraceptives and hormone replacement therapy cessation	Hormone therapy cessation		*CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP3A5, UGT1A1*
Abnormal Lipoprotein (a) levels	Modifiable	High lipoprotein (a) levels	Niacin (Nicotinic acid, vitamin B_3), Nutraceutical supplementation	Diet optimization	*LPA, LPL*	*ABCB1, CYP1A2, CYP2C9, CYP2D6, CYP3A4, CYP3A5, SLCO1B1, UGT1A1*
Excess homocysteine in blood	Modifiable	Hyperhomocysteinemia(>10 µmol/l)	Folic acid (Vitamin B_9)		*MTHFR*	*ABCB1, MTHFR*
Inflammation	Modifiable	Abnormal inflammation markers	Anti-inflammatory drugs	Neurological rehabilitation	*IL1B, IL6, IL6R, TNF*	*ABCB1, CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP3A5, G6PD, UGT1A1*
Abnormal blood coagulation	Modifiable	Thrombosis, Hemorrhage	Antithrombotic agents, Antihemorrhagics	See food interactions (vitamin K contributions)	*F2, F5, MTHFR*	*ABCB1, CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP3A5, G6PD, UGT1A1, VKORC1*
Stroke and/or cardiovascular disorder	Modifiable		Neurological protection	Neurological rehabilitation		
Advancing age	Non-modifiable	Elderly				
Heredity or family history	Non-modifiable	Genetic risk profile				
Gender	Non-modifiable	Male or female				
Ethnicity or race	Non-modifiable	Ethnic risk component				

ABCB1: ATP-binding cassette, sub-family B (MDR/TAP), member 1; *ACE*: Angiotensin I converting enzyme; *ADRB2*: Adrenoceptor beta 2, surface; *ADRB3*: Adrenoceptor beta 3; *AGT*: Angiotensinogen; *ANKK1*: Ankyrin repeat and kinase domain containing 1; *APOA2*: Apolipoprotein A-II; *APOA5*: Apolipoprotein A-V; *APOB*: Apolipoprotein B; *APOC3*: Apolipoprotein C-III; *APOE*: Apolipoprotein E; *CETP*: Cholesteryl ester transfer protein, plasma; *CHRNA4*: Cholinergic receptor, nicotinic, alpha 4; *CHRNA5*: Cholinergic receptor, nicotinic, alpha 5; *CHRNB2*: Cholinergic receptor, nicotinic, beta 2 (neuronal);*CLOCK*: Clock circadian regulator; *CYP1A1*: Cytochrome P450, family 1, subfamily A, polypeptide

1;*CYP1A2*: Cytochrome P450, family 1, subfamily A, polypeptide 2; *CYP2A6*: Cytochrome P450, family 2, subfamily A, polypeptide 6; *CYP2B6*: Cytochrome P450, family 2, subfamily B, polypeptide 6;*CYP2C19*: Cytochrome P450, family 2, subfamily C, polypeptide 19; *CYP2C9*: Cytochrome P450, family 2, subfamily C, polypeptide 9; *CYP2D6*: Cytochrome P450, family 2, subfamily D, polypeptide 6; *CYP2E1*: Cytochrome P450, family 2, subfamily E, polypeptide 1;*CYP3A4*: Cytochrome P450, family 3 subfamily A, polypeptide 4; *CYP3A5*: CytochromeP450, family 3 subfamily A, polypeptide 5; *DBH*: Dopamine Beta-Hydroxylase; *DDC*: Dopa decarboxylase (aromatic L-amino acid decarboxylase); *DRD1*: Dopamine receptor D1; *DRD2*: Dopamine receptor D2; *DRD3*: Dopamine receptor D3;*F2*: Coagulation factor II, thrombin; *F5*: Coagulation factor V; *FABP2*: Fatty acid binding protein 2, intestinal; *FKBP5*: FK506 binding protein 5; *FTO*: Fat mass and obesity associated; *G6PD*: Glucose-6-phosphate dehydrogenase; *IL1B*: Interleukin 1 beta; *IL6*: Interleukin 6; *IL6R*: Interleukin 6 receptor; *KCNJ11*: Potassium channel, inwardly rectifying subfamily J, member 11; *LEP*: Leptin; *LEPR*: Leptin receptor; *LIPC*: Lipase, hepatic; *LPA*: Lipoprotein (a); *LPL*: Lipoprotein lipase; *MC4R*: Melanocortin 4 receptor; *MTHFR*: Methylenetetrahydrofolate reductase (NAD(P)H); *NAT2*: N-acetyltransferase 2 (arylamine N-acetyltransferase); *NOS3*: Nitric oxide synthase 3; *NPY*: Neuropeptide Y; *NRXN1*: Neurexin 1; *NTRK2*: Neurotrophic tyrosine kinase, receptor, type 2;*PLIN*: Perilipin 1; *PPARG*: Peroxisome proliferator-activated receptor gamma; *SERT*: Serotonin (5-HT) Transporter; *SLC2A2*: Solutecarrier family 2 (facilitated glucose transporter), member 2; *SLC30A8*: Solute carrier family 30 (zinc transporter), member 8; *SLCO1B1*: Solute carrier organic anion transporter family, member 1B1; *SNCA*: Synuclein, alpha (non A4 component of amyloid precursor);*TAS1R2*: Taste receptor, type 1, member 2; *TCF7L2*: Transcription factor 7-like 2 (T-cell specific, HMG-box); *TNF*: Tumor necrosis factor; *UCP2*: Uncoupling protein 2 (mitochondrial, proton carrier); *UGT1A1*: UDP glucuronosyltransferase 1 family, polypeptide A1; *VKORC1*: Vitamin K epoxide reductase complex, subunit 1.

Several studies performed across various ethnicities and populations demonstrate a strong association between smoking and stroke risk, with current smokers having at least a two- to fourfold increased risk of stroke compared with lifelong nonsmokers or individuals who had quit smoking more than 10 years prior[48-53]. Even more, there is substantial scientific evidence showing a strong dose-response relationship between smoking and the risk of stroke [54].

Physical Inactivity

Physical activity improves risk factors for cardiovascular disease (such as high blood pressure and high cholesterol) and reduces the likelihood of diseases related to cardiovascular disease, including coronary heart disease, stroke, type 2 diabetes mellitus, and sudden heart attacks [55].

Chronic physical inactivity contributes to a poor level of cardiorespiratory fitness, which is a stronger predictor of adverse cardiovascular outcomes than traditional risk factors. Although both physical activity and cardiorespiratory fitness are inversely related to the risk of cardiovascular disease and other clinical outcomes, they are in part distinct measures in the assessment of cardiovascular disease risk [56].

Overweight and Obesity

Overweight and obesity are typically classified by use of BMI (Body Mass Index) cutoffs, but variations in body fat distribution (larger waist circumference) are also associated with increased cardiovascular risk [57]. Overweight and obesity are major risk factors for cardiovascular disease, including coronary heart disease, stroke [58,59], atrial fibrillation [60], venous thromboembolism [61], and congestive heart failure. BMI<25 kg/m2 (for adults aged ≥20 years) is considered as healthy normal weight.

Obesity is associated with subclinical atherosclerosis including coronary artery disease and carotid intima-media thickness, and this association persists after adjustment for cardiovascular risk factors, as shown in MESA (Multi-Ethnic Study of Atherosclerosis) [62].

A systematic review of prospective studies examining overweight and obesity as predictors of major stroke subtypes in >2 million participants over ≥4 years found an adjusted RR for ischemic stroke of 1.22 (95% CI, 1.05-1.41) in overweight individuals and an RR of 1.64 (95% CI, 1.36-1.99) for obese individuals relative to normal-weight individuals. RRs for hemorrhagic stroke were 1.01 (95% CI, 0.88-1.17) and 1.24 (95% CI, 0.99-1.54) for overweight and obese individuals, respectively. These risks were graded with increasing BMI and were independent of age, lifestyle, and other cardiovascular risk factors [63].

A recent meta-analysis of 15 prospective studies demonstrated the increased risk for Alzheimer's disease or vascular dementia and any dementia was 1.35 and 1.26 for overweight, respectively, and 2.04 and 1.64 for obesity, respectively [64].

Unhealthy Diet

Dietary habits affect multiple cardiovascular risk factors: blood pressure, cholesterol, glucose, obesity, inflammation, cardiac arrhythmias, endothelial cell function, triglycerides, lipoprotein (a), and heart rate.

A DASH (Dietary Approaches to Stop Hypertension) dietary pattern with low sodium reduced SBP by 7.1 mmHg in adults without hypertension and by 11.5 mm Hg in adults with hypertension [65].

In a meta-analysis of 60 randomized controlled feeding trials, consumption of 1% of calories from saturated fat in place of carbohydrate raised LDL cholesterol concentrations but also raised HDL cholesterol and lowered triglycerides, with no significant effects on apolipoprotein B concentrations [66].

In a pooled analysis of 25 randomized trials totaling 583 men and women both with and without hypercholesterolemia, nut consumption significantly improved blood lipid levels [67].

In meta-analyses of prospective cohort studies, each daily serving of fruits or vegetables was associated with a 4% lower risk of coronary heart disease (RR, 0.96, 95% CI, 0.93-0.99) and a 5% lower risk of stroke (RR, 0.95, 95% CI, 0.92-0.97) [68,69].

In the WHI (Women's Health Initiative) randomized clinical trial (N=48835), reduction of total fat consumption from 37.8% energy (baseline) to 24.3% energy (at 1 year) and 28.8% energy (at 6 years) had no effect on incidence of coronary heart disease (RR, 0.98, 95% CI, 0.88-1.09), stroke (RR, 1.02, 95% CI, 0.90-1.15), or total cardiovascular disease (RR, 0.98, 95% CI, 0.92-1.05) over a mean of 8.1 years [70].

In a cohort of 380296 US men and women, greater versus lower adherence to a Mediterranean dietary pattern, characterized by higher intakes of vegetables, legumes, nuts, fruits, whole grains, fish, and unsaturated fat and lower intakes of red and processed meat, was associated with a 22% lower cardiovascular mortality (RR, 0.78, 95% CI, 0.69-0.87) [71]. Similar findings have been seen for the Mediterranean dietary pattern and risk of incident coronary heart disease and stroke [72] and for the DASH-type dietary pattern [73].

Diabetes Mellitus (DM)

Epidemiological studies have shown that patients with diabetes mellitus and glucose intolerance are at increased risk for cardiovascular disease and stroke [74]. Untreated fasting blood glucose levels <100 mg/dL isan indisputable feature for ahealthy cardiovascular profile [75].

At least 68% of people >65 years of age with DM die of some form of heart disease and 16% die of stroke. The heart disease death rates among adults with DM are 2 to 4 times higher than the rates for adults without DM [76].

A meta-analysis of prospective randomized controlled trials of interventions that targeted people with pre-diabetes revealed a 24% relative risk reduction in fatal and nonfatal strokes (HR, 0.76, 95% CI, 0.58-0.99) [77].

In people with a history of TIA or minor stroke, impaired glucose tolerance nearly doubled the stroke risk compared with those with normal glucose levels and tripled the risks for those with DM [78].

The ACCORD study showed that in patients with type 2 DM, targeting SBP to <120 mmHg did not reduce the rate of cardiovascular events compared with subjects in whom the SBP target was <140 mm Hg, except for the end point of stroke, for which intensive therapy reduced the risk of any stroke (HR, 0.59, 95% CI, 0.39-0.89) and nonfatal stroke (HR, 0.63, 95% CI, 0.41-0.96) [79].

Alcohol

The complex relationship between alcohol consumption and stroke includes both benefits and risks. Regular light-to-moderate consumption of alcohol seems to decrease the risk for ischemic stroke by reducing atherothrombotic events, but the underlying mechanism is still unclear. Regular heavy drinking increases the risk for both hemorrhagic and ischemic strokes. Alcoholic cardiomyopathy is a cause of cardioembolic brain infarction. Cardiac arrhythmias caused by regular heavy drinking or binge drinking can precipitate thrombus formation and propagate already existing thrombi from the heart. The maintenance of high blood pressure by heavy drinking may promote cerebral arterial degeneration. Acute increases in systolic blood pressure and/or alterations in cerebral arterial tone could serve as mechanisms triggering hemorrhagic strokes during alcoholic intoxication [80].

Psychosocial Stress and Mental Disorders

The National Health and Nutrition Examination Survey showedthat higher levels of anxiety and depressive symptoms were associated with increased risk of incident stroke after adjustment for demographic, cardiovascular, and behavioral risk factors (HR, 1.14, 95% CI, 1.03-1.25) [81].

In the Chicago Health and Aging Project, higher psychological distress was associated with higher stroke mortality (HR, 1.29, 95% CI, 1.10-1.52) and incident hemorrhagic strokes (HR, 1.70, 95% CI, 1.28-2.25) [82].

The Australian Longitudinal Study on Women's Healthshowed that depression was associated with a nearly 2-fold increased odd of stroke after adjustment for age, socioeconomic status, lifestyle, and physiological risk factors (OR, 1.94, 95% CI, 1.37-2.74) [83].

In a meta-analysis of 17 community-based or population-based prospective studies people with a history of depression experienced a 34% higher risk for the development of subsequent stroke after adjustment for potential confounding factors (RR, 1.34, 95% CI, 1.17-1.54) [84].

A meta-analysis of 28 prospective cohort studies comprising 317540 participants with a follow-up period that ranged from 2 to 29 years found that depression was prospectively associated with an increased risk of total stroke (HR, 1.45, 95% CI, 1.29-1.63), fatal stroke (HR, 1.55, 95% CI, 1.25-1.93), and ischemic stroke (HR, 1.25, 95 CI, 1.11-1.40) [85].

Lipoprotein(a)

Lipoprotein(a) is formed by joining a lipoprotein that is structurally similar to LDL in protein and lipid compositionto a carbohydrate-rich, hydrophilic protein called apo(a). The physiology and function of Lp(a) are still poorly understood, but the apolipoprotein(a) molecule demonstrates high sequence homology (75-90%) with plasminogen [86]. This suggests that Lp(a) might contribute to the thrombotic, as well as to the atherogenic, aspects of IHD [87].

The meta-analysis made by Craig et al. [88] showed that Lp(a) is an independent prospective risk factor for IHD. This finding, together with evidence for a dose-response relationship between Lp(a) and IHD, provides support for a causative role for Lp(a) in the development of atherosclerosis.

In the PRIME study [89] Lp(a) appeared significantly related to coronary heart disease development as a significant risk factor (P<0.0006).The PRIME study evidence that subjects with levels of Lp(a) in the highest quartile had more than 1.5 times the risk than subjects in the lowest quartile.

Homocysteine

Impaired homocysteine metabolism has been implicated as a factor in atherosclerosis, cerebrovascular disease, and peripheral vascular disease and several case-control and cohort studies have linked hyperhomocysteinemia with coronary heart disease [90-92]. The British Regional Heart Study showedthat homocysteine levels were significantly (P=0.004) higher in patients with stroke [93].

The Supplementation with Antioxidant Vitamins and Minerals Study suggested that to control homocysteine, decreasing coffee and alcohol consumption may be important in women, whereas increasing physical activity, dietary fiber, and folate intake may be important in men [94].

Atrial Fibrillation

Atrial fibrillation(AF) is a powerful risk factor for stroke, independently increasing risk 5-fold throughout all ages. The percentage of strokes attributable to AF increases steeply from 1.5% at 50 years of age to 23.5% at 80 years of age [95,96].

Advancing Age

Stroke patients >85 years of age make up 17% of all stroke patients [97]. Over the next 40 years (2010-2050), the number of incident strokes is expected to more than double, with the majority of the increase among the elderly (aged ≥75 years) and minority groups [98].

Heredity or Family History

Heritability is the ratio of genetically caused variation to the total variation of a trait or measure.Genetic markers discovered thus far have not been shown to add to cardiovascular risk prediction tools beyond current models that incorporate family history [99]. Genetic markers also have not been shown to improve prediction of subclinical atherosclerosis beyond traditional risk factors [100].

In the Framingham Heart Study, a documented parental ischemic stroke by the age of 65 years was associated with a 3-fold increase in ischemic stroke risk in offspring, even after adjustment for other known stroke risk factors. The absolute magnitude of the increased risk was greatest in those in the highest quintile of the Framingham Risk Score. By age 65 years, people in the highest Framingham Risk Score quintile with an early parental ischemic stroke had a 25% risk of stroke compared with a 7.5% risk of ischemic stroke for those without such a history [101].

The 9p21.3 region polymorphisms [102-104] and the histone deacetylase 9 (*HDAC9*) on chromosome 7p21.1[104,105] showed evidence of correlation with ischemic stroke.

Gender

The Framingham Heart Study reveals that women with natural menopause before 42 years of age had twice the ischemic stroke risk of women with natural menopause after 42 years of age [106].

Randomized clinical trial data indicate that the use of estrogen plus progestin, as well as estrogen alone, increases stroke risk in postmenopausal, generally healthy women and provides no protection for postmenopausal women with established CHD [107-110] and recent stroke or TIA [111].

Low-estrogen-dose oral contraceptives are associated with a 93% increased risk of ischemic stroke, but the absolute increased risk is small (4.1/100000 ischemic strokes in nonsmoking, normotensive women) [112,113].

Migraine with aura is associated with ischemic stroke inyounger women, particularly if they smoke or use oral contraceptives. The combination of all 3 factors increases therisk 9-fold compared with women without any of these factors [114,115].

Preeclampsia is a risk factor for ischemic stroke remote from pregnancy [116]. The increase in stroke risk related to preeclampsia may be mediated by later risk of hypertension and diabetes [117].

Genetic Markers in Cerebrovascular Disorders

Due to the interaction between genes and environment, complex diseases may be forestalled by acting on the environmental factors with an appropriate prevention plan. Knowledge of the genes involved in the development of these diseases (**Table 2**) enables us to make certain predictions regarding the risks, susceptibilities or resistance to developing them [118]. Genetic testing for

cerebrovascular diseases establishes the susceptibility, risk or probability of an individual suffering the disease. For this reason, the results of the tests merely indicate that a person may have a greater probability, risk or susceptibility to suffering the disease than the population at large, but it does not mean that the person will necessarily suffer this disease, as this risk is influenced by other variables, such as external conditioning factors.

Table 2. Biomarker candidates as cerebrovascular risk predictors.

Symbol	Gene	Locus	dbSNP	Polymorphism
ABCA1	ATP-binding cassette, subfamily A, member 1	9q22-q31		T-477C
ABCA1	ATP-binding cassette, subfamily A, member 1	9q22-q31	rs2230806	G1051A (Arg219Lys)
ABCA1	ATP-binding cassette, subfamily A, member 1	9q22-q31	rs4149313	A2583G (Ile823Met)
ACE	Angiotensin I- converting enzyme	17q23	rs4291	A-240T
ACE	Angiotensin I- converting enzyme	17q23		Intron 16 Alu 287bp I/D
ADRB1	Beta-1-adrenergic receptor	10q24-q26	rs1801253	G1165C (Gly389Arg)
ADRB2	Beta-2-adrenergic receptor HA	5q32-q34	rs1042713	A46G (Arg16Gly)
ADRB2	Beta-2-adrenergic receptor	5q32-q34	rs1042714	C79G (Gln27Glu)
ADRB3	Beta-3-adrenergic receptor	8p12-p11.2	rs4994	T190C (Trp64Arg)
AGT	Angiotensinogen	1q42-q43	rs5051	G-6A
AGT	Angiotensinogen	1q42-q43		M235T
AGT	Angiotensinogen	1q42-q43		T174M
AGTR1	Angiotensin receptor 1	3q21-q25	rs1492078	C-535T
AGTR1	Angiotensin receptor 1	3q21-q25	rs5186	A1166C
AGTR1	Angiotensin receptor 1	3q21-q25	rs12721226	G→A (Ala163Thr)
AGTR1	Angiotensin receptor 1	3q21-q25	rs12721225	G→T (Ala244Ser)
AGTR1	Angiotensin receptor 1	3q21-q25	rs1801021	A→C (Thr336Pro)
AGTR2	Angiotensin II receptor, type 2	Xq22-q23	rs1403543	G1675A
AGTR2	Angiotensin II receptor, type 2	Xq22-q23	rs11091046	C3123A
ALOX5	Arachidonate 5-lipoxygenase	10q11.2	rs12762604	C3175G
ALOX5	Arachidonate 5-lipoxygenase	10q11.2		Sp1 STR
ALOX5	Arachidonate 5-lipoxygenase	10q11.2	rs12762604	G→A (Phe42Leu)
ALOX5	Arachidonate 5-lipoxygenase	10q11.2	rs2228065	G→A (Glu254Lys)
ANXA5	Annexin A5	4q26-q28	rs11575945	C-1T
AP2M1	Adaptor-related protein complex 2, MU-1 subunit	3q28	rs1501299	G62T
APOA1	Apolipoprotein A-I	11q23	rs670	G-75A
APOA1	Apolipoprotein A-I	11q23	rs5070	T84C
APOA5	Apolipoprotein A-V	11q23	rs662799	T-1131C
APOB	Apolipoprotein B	2p24		C2488T (XbaI)
APOB	Apolipoprotein B	2p24		G10708A (Arg3500Gln)
APOB	Apolipoprotein B	2p24		C10800T (Arg3531Cys)
APOC3	Apolipoprotein C-III	11q23	rs2854117	C-482T
APOC3	Apolipoprotein C-III	11q23	rs4520	C1100T
APOC3	Apolipoprotein C-III	11q23		C3175G (S1/S2)
APOE	Apolipoprotein E	19q13.2	rs405509	G-219T
APOE	Apolipoprotein E	19q13.2	rs429358	T3932C (Cys112Arg)
APOE	Apolipoprotein E	19q13.2	rs7412	C4070T (Arg158Cys)
CAPN10	Calpain 10	2q37.3	rs3792267	G4852A
CCL2	Chemokine, CC motif, ligand 2	17q11.2-q12		A-2518G
CCL5	Chemokine, CC motif, ligand 5	17q11.2-q12	rs2280788	C-28G
CCL5	Chemokine, CC motif, ligand 5	17q11.2-q12	rs2107538	G-403A
CCL11	Chemokine, CC motif, ligand 11	17q21.1-q21.2	rs3744508	G→A (Ala23Thr)
CCND1	Cyclin D1	11q13	rs2220247	G→T (Ala30Ser)
CCR2	Chemokine, CC motif, receptor 2	3p21	rs1799864	G190A (Val64Ile)
CCR5	Chemokine, CC motif, receptor 5	3p21	rs1799987	G59029A
CD14	Monocyte differentiation antigen CD14	5q31.1	rs2569190	C-260T
CD36	CD36 antigen	7q11.2	rs1049673	G30294C
CD36	CD36 antigen	7q11.2		C12293T (Pro90Ser)

CD40	CD40 antigen	20q12-q13.2		A455T
CETP	Cholesteryl ester transfer protein, plasma	16q21		G279A (TaqB1/B2)
CETP	Cholesteryl ester transfer protein, plasma	16q21	rs1800775	C-629A
CETP	Cholesteryl ester transfer protein, plasma	16q21	rs5882	A1061G (Ile405Val)
COL1A2	Collagen, type I, alpha-2	7q22.1	rs42524	G→C (Ala459Pro)
COL3A1	Collagen, type III, alpha-1	2q31	rs1800255	G2209A (Ala698Thr)
COL3A1	Collagen, type III, alpha-1	2q31	rs2271683	A3730G (Ile1205Val)
CRP	C-reactive protein, pentraxin-related	1q21-q23	rs1130864	C1444T
CX3CR1	Chemokine, CX3C motif, receptor 1	3pter-p21	rs3732378	C926T (Thr280Met)
CXCL16	Chemokine, CXC motif, ligand 16	17p13	rs2277680	C→T (Ala181Val)
ELN	Elastin	7q11.2	rs2071307	G1264A (Gly422Ser)
EPHX2	Epoxide hydrolase 2, cytosolic	8p21-p12	rs751141	G→A (Arg287Gln)
ESR1	Estrogen receptor 1	6q25.1	rs2071454	T-1989G
F3	Coagulation factor III	1p22-p21	rs1361600	A-603G
F7	Factor VII	13q34	rs6046	G11496A (Arg353Gln)
F12	Factor XII	5q33-qter	rs17876008	C46T
FABP2	Fatty acid-binding protein 2	4q28-q31	rs1799883	G2445A (Ala54Thr)
FBN1	Fibrillin 1	15q21.1	rs25458	T1875C
FGB	Fibrinogen, B beta polypeptide	4q28	rs1800790	G-455A
FGB	Fibrinogen, B beta polypeptide	4q28	rs4220	G8059A (Arg448Lys)
HDAC9	Histone deacetylase 9	7p21.1		
HNF4A	Hepatocyte nuclear factor 4-alpha	20q12-q13.1	rs2425640	A→G
ICAM1	Intercellular adhesion molecule 1	19p13.3-p13.2	rs5498	G1462A (Glu469Lys)
IGF2R	Insulin-like growth factor II receptor	6q26	rs629849	A5002G (Arg1619Gly)
IL1B	Interleukin 1-beta	2q14	rs16944	C-511T
IL1RN	Interleukin 1 Receptor Antagonist	2q14.2		IL1RN*2 VNTR
IL6	Interleukin 6	7p21		G-174C
IL6	Interleukin 6	7p21	rs1800796	G-572C
IL10	Interleukin 10	1q31-q32		A-1082G
IL10	Interleukin 10	1q31-q32	rs1800871	T-819C
IL10	Interleukin 10	1q31-q32	rs1800872	A-592C
INS	Insulin	11p15.5	rs689	T-23A
INSR	Insulin receptor	19p13.2	rs2860172	C7067365A
IPF1	Insulin promoter factor 1	13q12.1	(S82168)	-108/3G→4G
IRS1	Insulin receptor substrate 1	2q36	rs1801277	A3694G (Ser892Gly)
IRS1	Insulin receptor substrate 1	2q36	rs1801278	G3931A (Gly972Arg)
ITGA2	Integrin, alpha-2	5q23-q31	rs10471371	A1648G (Lys505Glu)
ITGB2	Integrin, beta-2	21q22.3	rs235326	C1323T
LDLR	Low density lipoprotein receptor	19p13.2	rs11669576	G1184A (Ala370Thr)
LMNA	Lamin A/C	1q21.2	rs11549669	A→G (Glu2Gly)
LPL	Lipoprotein lipase	8p22	rs328	C1595G (Ser447Stop)
MMP1	Matrix metalloproteinase 1	11q22-q23	rs1799750	-1607/1G→2G
MMP1	Matrix metalloproteinase 1	11q22-q23		A-519G
MMP1	Matrix metalloproteinase 1	11q22-q23		T-340C
MMP2	Matrix metalloproteinase 2	16q13	rs243865	C-1306T
MMP3	Matrix metalloproteinase 3	11q23	rs3025058	-1171/5A→6A
MMP3	Matrix metalloproteinase 3	11q23		indel-1612A
MMP3	Matrix metalloproteinase 3	11q23		A-709G
MMP3	Matrix metalloproteinase 3	11q23	rs679620	A→G (Lys45Glu)
MMP3	Matrix metalloproteinase 3	11q23	rs11606831	A→C (His113Pro)
MMP9	Matrix metalloproteinase 9	20q11.2-q13.1	rs3918242	C-1562T
MMP9	Matrix metalloproteinase 9	20q11.2-q13.1	rs2664538	G855A (Arg279Gln)
MMP12	Matrix metalloproteinase 12	11q22.2-q22.3	rs2276109	A-82G

MPO	Myeloperoxidase	17q23.1	(NT_035425)	G-463A
MPO	Myeloperoxidase	17q23.1	(AH002972)	G-129A
MTHFR	5,10-Methylenetetrahydrofolate reductase	1p36.3	rs1801133	C677T (Ala222Val)
NFKB	Nuclear Factor Kappa B, subunit 1	4q23-q24		indel-94ATTG
NOS3	Nitric oxide synthase 3	7q36		G37498A
NOS3	Nitric oxide synthase 3	7q36	rs2070744	T-786C
NOS3	Nitric oxide synthase 3	7q36		4a/4b
NOS3	Nitric oxide synthase 3	7q36		G894T
NPY	Neuropeptide Y	7p15.1		T1128C (L7P)
OLR1	Low density lipoprotein, oxidized, receptor 1	12p13-p12	rs11053646	G501C (Lys167Asn)
P2RY12	Purinergic receptor P2Y, G protein-coupled, 12	3q24-q25	(NC_000003)	T744C
PAI1	Plasminogen activator inhibitor 1	7q21.3-q22	rs1799768	-668/4G→5G
PAI1	Plasminogen activator inhibitor 1	7q21.3-q22	rs2227647	A→C (His25Pro)
PAI1	Plasminogen activator inhibitor 1	7q21.3-q22	rs2227669	G→A (Arg209His)
PAI1	Plasminogen activator inhibitor 1	7q21.3-q22	rs13306846	A→G (Tyr243Cys)
PAX4	Paired box gene 4	7q32	(AF043978)	C567T (Arg121Trp)
PECAM1	Platelet-endothelial cell adhesion molecule 1	17q23	rs668	C1454G (Leu125Val)
PECAM1	Platelet-endothelial cell adhesion molecule 1	17q23	rs1131012	G2201A (Gly670Arg)
PIK3R1	Phosphatidylinositol 3-kinase, regulatory, 1	5q13	rs3730089	G1020A (Met326Ile)
PON1	Paraoxonase 1	7q21.3	rs705381	G-162A
PON1	Paraoxonase 1	7q21.3	rs13306698	A532G (Arg160Gly)
PON1	Paraoxonase 1	7q21.3	rs662	G584A (Gln192Arg)
PON2	Paraoxonase 2	7q21.3	rs11545941	C475G (Ala148Gly)
PPARD	Peroxisome proliferator-activated receptor-delta	6p21.2-p21.1	rs2016520	T294C
PPARG	Peroxisome proliferator-activated receptor-gamma	3p25	rs10865710	C-681G
PPARG	Peroxisome proliferator-activated receptor-gamma	3p25	rs1801282	C34G (Pro12Ala)
PPARGC 1	Peroxisome proliferator-activated receptor-gamma, coactivator 1	4p15.1	rs8192678	G1564A (Gly482Ser)
SAH	Hypertension-associated SA, rat, homolog of	16p13.11	rs13306607	A→G (-7 from exon 13)
SELE	Selectin E	1q23-q25	rs5361	A561C (Ser128Arg)
SELP	Selectin P	1q23-q25	rs6136	A37674C (Thr715Pro)
SELP	Selectin P	1q23-q25	rs6133	G→T (Val640Leu)
SRA	Scavenger Receptor A1	8p22		C877T
SRB1	Scavenger Receptor B1	12q24.31		G2S
SREBF1	Sterol regulatory element-binding transcription factor 1	17p11.2	(AX977070)	indel-36G
TCF1	Transcription factor 1	12q24.2	rs1800574	C→T (Ala98Val)
TGFB1	Transforming growth factor, beta-1	19q13.1	rs1800469	C-509T
TGFBR2	Transforming growth factor-beta receptor, type II	3p22	rs2228048	C1167T (Asn389Asn)
THBD	Thrombomodulin	20p11.2	rs1042579	C2136T (Ala455Val)
THBS2	Thrombospondin II	6q27	rs8089	T3949G
THBS4	Thrombospondin IV	5q13	rs1866389	G1186C (Ala387Pro)
THPO	Thrombopoietin	3q26.3-q27	rs6141	A5713G
TLR4	Toll-like receptor 4	9q32-q33		A896G
TLR4	Toll-like receptor 4	9q32-q33	rs4986790	A2326G (Asp299Gly)
TNF	Tumor necrosis factor	6p21.3	rs1800630	C-863A
TNF	Tumor necrosis factor	6p21.3	rs1799724	C-850T
TNF	Tumor necrosis factor	6p21.3	rs361525	G-238A
TNFRSF1 A	Tumor necrosis factor receptor 1	12p13.2		R92Q
TNFSF4	Tumor necrosis factor ligand superfamily, member 4	1q25	rs3850641	A→G
VEGF	Vascular endothelial growth factor	6p12		A-2518G
VEGF	Vascular endothelial growth factor	6p12	rs3025039	C936T

ABCA1: ATP-binding cassette, subfamily A, member 1; *ACE:* Angiotensin I- converting enzyme; *ADRB1:* Beta-1-adrenergic receptor; *ADRB2:* Beta-2-adrenergic receptor HA; *ADRB3:* Beta-3-adrenergic receptor; *AGT:* Angiotensinogen; *AGTR1:* Angiotensin receptor 1; *AGTR2:* Angiotensin II receptor, type 2; *ALOX5:* Arachidonate 5-lipoxygenase; *ANXA5:* Annexin A5; *AP2M1:* Adaptor-related protein complex 2, MU-1 subunit; *APOA1:* Apolipoprotein A-I; *APOA5:* Apolipoprotein A-V; *APOB:* Apolipoprotein B; *APOC3:* Apolipoprotein C-III; *APOE:* Apolipoprotein E; *CAPN10:* Calpain 10; *CCL2:* Chemokine, CC motif, ligand 2; *CCL5:* Chemokine, CC motif, ligand 5; *CCL11:* Chemokine, CC motif, ligand 11; *CCND1:* Cyclin D1; *CCR2:* Chemokine, CC motif, receptor 2; *CCR5:* Chemokine, CC motif, receptor 5; *CD14:* Monocyte differentiation antigen CD14; *CD36:* CD36 antigen; *CD40:* CD40 antigen; *CETP:* Cholesteryl ester transfer protein, plasma; *COL1A2:* Collagen, type I, alpha-2; *COL3A1:* Collagen, type III, alpha-1; *CRP:* C-reactive protein, pentraxin-related; *CX3CR1:* Chemokine, CX3C motif, receptor 1; *CXCL16:* Chemokine, CXC motif, ligand 16; *ELN:* Elastin; *EPHX2:* Epoxide hydrolase 2, cytosolic; *ESR1:* Estrogen receptor 1; *F3:* Coagulation factor III; *F7:* Factor VII; *F12:* Factor XII; *FABP2:* Fatty acid-binding protein 2; *FBN1:* Fibrillin 1; *FGB:* Fibrinogen, B beta polypeptide; *HDAC9*: Histone deacetylase 9; *HNF4A:* Hepatocyte nuclear factor 4-alpha; *ICAM1:* Intercellular adhesion molecule 1; *IGF2R:* Insulin-like growth factor II receptor; *IL1B:* Interleukin 1-beta; *IL1RN:* Interleukin 1 Receptor Antagonist; *IL6:* Interleukin 6; *IL10:* Interleukin 10; *INS:* Insulin; *INSR:* Insulin receptor; *IPF1:* Insulin promoter factor 1; *IRS1:* Insulin receptor substrate 1; *ITGA2:* Integrin, alpha-2; *ITGB2:* Integrin, beta-2; *LDLR:* Low density lipoprotein receptor; *LMNA:* Lamin A/C; *LPL:* Lipoprotein lipase; *MMP1:* Matrix metalloproteinase 1; *MMP2:* Matrix metalloproteinase 2; *MMP3:* Matrix metalloproteinase 3; *MMP9:* Matrix metalloproteinase 9; *MMP12:* Matrix metalloproteinase 12; *MPO:* Myeloperoxidase; *MTHFR:* 5,10-Methylenetetrahydrofolate reductase; *NFKB:* Nuclear Factor Kappa B, subunit 1; *NOS3:* Nitric oxide synthase 3; *NOS3:* Nitric oxide synthase 3; *NPY:* Neuropeptide Y; *OLR1:* Low density lipoprotein, oxidized, receptor 1; *P2RY12:* Purinergic receptor P2Y, G protein-coupled, 12; *PAI1:* Plasminogen activator inhibitor 1; *PAX4:* Paired box gene 4; *PECAM1:* Platelet-endothelial cell adhesion molecule 1; *PIK3R1:* Phosphatidylinositol 3-kinase, regulatory, 1; *PON1:* Paraoxonase 1; *PON2:* Paraoxonase 2; *PPARD:* Peroxisome proliferator-activated receptor-delta; *PPARG:* Peroxisome proliferator-activated receptor-gamma; *PPARGC1:* Peroxisome proliferator-activated receptor-gamma, coactivator 1; *SAH:* Hypertension-associated SA, rat, homolog of; *SELE:* Selectin E; *SELP:* Selectin P; *SRA:* Scavenger Receptor A1; *SRB1:* Scavenger Receptor B1; *SREBF1:* Sterol regulatory element-binding transcription factor 1; *TCF1:* Transcription factor 1; *TGFB1:* Transforming growth factor, beta-1; *TGFBR2:* Transforming growth factor-beta receptor, type II; *THBD:* Thrombomodulin; *THBS2:* Thrombospondin II; *THBS4:* Thrombospondin IV; *THPO:* Thrombopoietin; *TLR4:* Toll-like receptor 4; *TNF:* Tumor necrosis factor; *TNFRSF1A:* Tumor necrosis factor receptor 1; *TNFSF4:* Tumor necrosis factor ligand superfamily, member 4; *VEGF:* Vascular endothelial growth factor.

Genetic tests of this type are integrated in multigenic panels, as development of these diseases is not caused by a single gene, but by the interaction of a number of genes (**Figure 1**). These genetic panels study different genetic polymorphisms or variations in the DNA sequence, which are involved in the development, prognosis and evolution of these pathologies, and represent a key tool in medical practice.

Cerebrovascular Risk and Lipid Metabolism

Among the many genetic and environmental risk factors that have been identified by epidemiologic studies, elevated levels of serum cholesterol are probably unique in being sufficient to drive the development of atherosclerosis in humans and experimental animals, even in the absence of other known risk factors. Allelic variants for apolipoproteins such as APOB, APOCIII and APOE, as well as the cholesterol ester transfer protein (CETP) and the lipoprotein lipase (LPL) (**Table 3**), play a key role in lipoprotein metabolism and are linked to the development of atherosclerosis and increased vascular risk [119-126].

***APOA2*-Apolipoprotein A-II**

Apolipoprotein A2 (APOA2) is the second most abundant apolipoprotein in HDL [118]. Several studies describe relationships between *APOA2* variantswith insulin resistance, obesity and atherosclerosis susceptibility. Corella et al. [127,128] founded that*APOA2*-265CC* (rs5082) genotype is associated with obesity and increased appetite. Individual homozygous *APOA2*-265CC*with high saturated fat levels in their diet was strongly associated with increased BMI and obesity.

***APOA5*-Apolipoprotein A-V**

APOA5 is a component of high-density lipoprotein and play a role in triglyceride concentration, a risk factor for cardiovascular disease [118].*APOA5* haplotypes *2 and *3 are associated with increased plasma triglyceride concentrations [129,130]. Mutations in this gene have been associated with hypertriglyceridemia and hyperlipoproteinemia type 5 [131,132].

Moleres et al. [133] found that *APOA5*-644T* allele (rsrs662799) has been associated with greater weight andweight lossresponse.

The *APOA5*-1131C* was associated with an increased risk for the development of carotid plaque in patients with Type III hyperlipoproteinemia with an odds ratio of 3.69. Evaluation of the genotype distribution was compatible with an independent effect of APOA5. The development of atherosclerosis in patients with Type III

hyperlipoproteinemia is modulated by variation in the *APOA5* gene [134].

Both the natural variants of the apolipoprotein A5 (*APOA5*) and the glucokinase regulatory protein gene (*GCKR*) have been shown to associate with increased fasting triglyceride levels. Járomi et al. [135] investigated the possible association of the functional variants of these two genes with non-fasting triglyceride levels and their susceptibility nature in ischemic stroke. A total of 513 stroke patients and 172 healthy controls were genotyped. All the *APOA5* variants (T-1131C, IVS3+G476A, C56G, and T1259C) were associated with increased triglyceride levels in all stroke patients and controls, except for T1259C, they all conferred risk for the disease. No such association was found for the examined *GCKR* rs1260326 (C1337T) variant. They examined the effects of specific combinations of the *GCKR* rs1260326 and *APOA5* polymorphisms. These findings confirmed the previous results regarding the association of *APOA5* variants with triglyceride-level increase and stroke susceptibility of these alleles. By contrast, no association could be detected of the studied *GCKR* allele with triglyceride levels or with the susceptibility of stroke in the same cohort of patients. The effect of *APOA5* did not change significantly when specific combinations of the two genes were present.

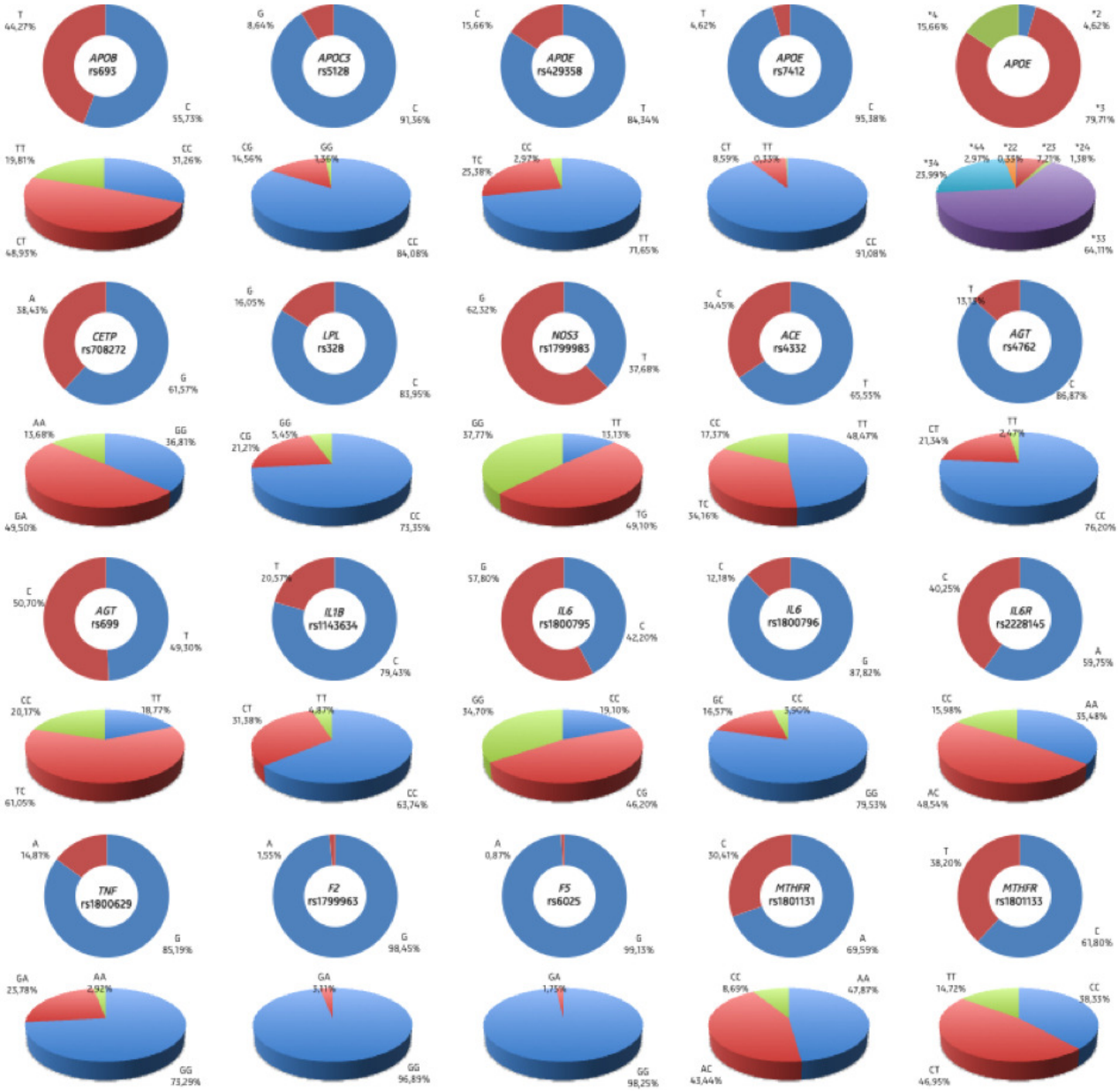

Figure 1. Impact of vascular genetic risk polymorphisms in Spanish population (N=2459)

Table 3. Polymorphisms related with cerebrovascular risk and lipid metabolism

Gene Symbol	Gene Name	Locus	dbSNP	Polymorphism
APOA2	Apolipoprotein A-II	1q23.3	rs5082	c.-265T>C
APOA5	Apolipoprotein A-V	11q23.3	rs662799	c.-644C>T
APOB	Apolipoprotein B	2p24.1	rs693	c.2488C>T
APOC3	Apolipoprotein C-III	11q23.3	rs5128	c.3175C>G; S1/S2
APOE	Apolipoprotein E	19q13.2	rs429358 rs7412	c.3932T>C; p.Cys112Arg c.4070C>T; p.Arg158Cys
CETP	Cholesteryl ester transfer protein, plasma	16q13	rs708272	c.+279G>A
FABP2	Fatty acid binding protein 2, intestinal	4q26	rs1799883	c.163G>A; p.Ala54Thr
LPL	Lipoprotein lipase	8p21.3	rs328	c.1421C>G; S447X

APOA2: Apolipoprotein A-II; *APOA5*: Apolipoprotein A-V; *APOB*: Apolipoprotein B; *APOC3*: Apolipoprotein C-III; *APOE*: Apolipoprotein E; *CETP*: Cholesteryl ester transfer protein, plasma; *FABP2*: Fatty acid binding protein 2, intestinal; *LPL*: Lipoprotein lipase.

APOB-Apolipoprotein B

Apolipoprotein B is the main apolipoprotein of chylomicrons and low-density lipoproteins [118]. Increased levels of ApoB are directly associated with atherogenic lipoproteins, VLDL, IDL and LDL. It is synthesized primarily in the liver and intestine. The *APOB*7545C* allele (rs693) is associated with lower levels of triglycerides, cholesterol and LDL cholesterol. However, individuals carrying the *APOB*7545T* allele respond better to a low fat diet, with significantly greater reduction in their levels of LDL and ApoB. Mutations in this gene or its regulatory region cause hypobetalipoproteinemia and hypercholesterolemia due to ligand-defective apoB, diseases affecting plasma cholesterol and apoB levels [136]. APOB K4154K homozygosity predicts a 3- to 5-fold reduction in risk of ischemic cerebrovascular disease and ischemic stroke. This may be explained by lower plasma levels of apolipoprotein B and LDL cholesterol caused by an increased catabolism of LDL particles, although another yet-unknown mechanism is also possible [137].

APOC3-Apolipoprotein C-III

Apolipoprotein C-III is a very low-density lipoprotein (VLDL) protein [118]. APOC3 inhibits lipoprotein lipase and hepatic lipase, it is thought to delay catabolism of triglyceride-rich particles [138]. An increase in ApoC-III levels induces the development of hypertriglyceridemia [139].

*APOC3*3175G* (S2) variant is associated with greater stability and higher levels of expression of ApoC-III, as it relates to increased risk of vascular disease due to its involvement in the metabolism of triglycerides [139]. Absence of APOC3, the natural LPL inhibitor, enhances fatty acid uptake from plasma triglycerides in adipose tissue, which leads to higher susceptibility to diet-induced obesity followed by more severe development of insulin resistance. Therefore, APOC3 is a potential target for treatment of obesity and insulin resistance.

APOE-Apolipoprotein E

ApoE, a main apoprotein of the chylomicron, binds to a specific receptor on liver cells and peripheral cells [118]. The E2 variant binds less readily. A defect in the receptor for ApoE on liver and peripheral cells might also lead to dysbetalipoproteinemia [140]. Although nearly every type III hyperlipoproteinemic person has the E2/E2 phenotype, 95 to 99% of persons with this phenotype do not have type III hyperlipoproteinemia nor do they have elevated plasma cholesterol levels. Rall et al. [141] founded that apoE2 of hypo-, normo-, and hypercholesterolemic subjects showed the same severe functional abnormalities.Type III hyperlipoproteinemia is strongly associated with the homozygous presence of the E2 allele of the *APOE* gene. However only about 10% of subjects with *APOE-2/2* genotype develop hyperlipidemia and it is therefore assumed that further genetic and environmental factors are necessary for the expression of disease [140].

Alterations in lipid metabolism associated with ApoEdysfunction may influence AD-related pathology. It has been proposed that the linkage of the APOE-4 to AD may represent dysfunction of the lipid transport system associated with compensatory sprouting and synaptic remodeling central to the AD process. ApoE may have a role in recycling cholesterol in membrane components in the brain where focal accumulation of ApoE in dystrophic axons is observed in cases of cerebrovascular disease. In AD there is a lower proportion of the HDL sub-fraction of largest particle size (HDL2b, mean diameter 10.57 nm) that contains the bulk of ApoE and a higher proportion of HDL3b of intermediate particle size (8.44 nm) than in the control population. No difference is observed in any of the HDL sub-fractions between AD with APOE-4 and those with APOE-2 or APOE-3. The differences between patients and controls are greater in APOE-4 carriers. There is no

major difference in the concentrations of the major lipoprotein fractions, suggesting that altered HDL sub-fraction profile in AD is a general feature of AD and is not a consequence of the APOE-4 phenotype. These data also indicate that the decreased concentration of HDL2b, which contains most of the HDL-associated ApoE, in AD may be related to the impaired ability of these patients to provide regenerating nerve cells with an adequate supply of cholesterol. The *APOE* gene promoter (-219G/T) polymorphism may influence the postprandial response of triacylglycerol-rich lipoproteins prolonging postprandial lipemia in subjects with the TT genotype [118].

CETP-Cholesteryl Ester Transfer Protein, Plasma

This gene encodes cholesterol ester transfer protein (CETP) that facilitates the exchange of triglycerides and cholesterol esters, stimulating the recovery of cholesterol [118]. The *CETP*+279A>G* polymorphism (rs708272) is associated with low levels of HDL cholesterol and high levels of plasma CETP activity (presence of the +279G allele), which contribute to an increased risk of cardiovascular disease [124].

CETP expression leads to a moderate increase in atherosclerosis in *apoE-0* and *LDLR-0* mice, and suggests a pro-atherogenic effect of CETP activity in metabolic settings in which clearance of remnants or LDL is severely impaired. However, apoA1 overexpression has more dramatic protective effects on atherosclerosis in *apoE-0* mice, which are not significantly reversedby concomitant expression of *CETP* [118].

FABP2-Fatty Acid Binding Protein 2, Intestinal

The intracellular fatty acid-binding proteins (FABPs) belong to a multigene family divided into at least three distinct types, namely hepatic, intestinal and cardiac types. They are thought to participate in the uptake, intracellular metabolism and transport of long-chain fatty acids. Fatty acid binding protein (FABP) is found in the small intestine epithelial cells where it strongly influences fat absorption and metabolism [118]. The *FABP2*163G>A*polymorphism(rs1799883, Ala54Thr) is associated with obesity, elevated BMI, increased abdominal fat, higher leptin levels, insulin resistance, higher insulin levels, and hypertriglyceridemia. *FABP2*163A* allele (54Thr variant) carriers have greater fat absorption and tend to have slower metabolism, leading to a tendency for weight gain, slower weight loss and difficulty in losing abdominal fat [142-146].

LPL-Lipoprotein Lipase

Lipoprotein lipase plays a key role in lipoprotein metabolism by hydrolyzing the triglycerides that are part of VLDL and the chylomicrons, and removing lipoproteins from the circulation [118]. LPL influences the interaction of atherogenic lipoproteins with cell surface receptors and the vascular wall [125]. Recent studies link the *LPL*1421C>G* polymorphism (rs328) [Ser447Stop] (truncated protein of 446 amino acids instead of 448) with a lower risk of coronary heart disease due to its relationship with increased HDL and decreased triglycerides [126]. Therefore, the 447X variant has higher enzymatic activity and should therefore have a protective effect against the development of atherosclerosis and subsequent coronary artery disease (CAD).

Genome-wide association studies in European Americans have reported several SNPs in the lipoprotein lipase gene associated with plasma levels of high-density lipoprotein cholesterol (HDL-C) and triglycerides [118]. However, the influences of the lipoprotein lipase SNPs on longitudinal changes of these lipids have not been systematically examined. On the basis of data from 2045 African-Americans and 2116 European Americans in the Coronary Artery Risk Development in Young Adults study, cross-sectional and longitudinal associations of lipids with 8 lipoprotein lipase SNPs, including 2 that had been reported in genome-wide association studies, were investigated [147]. Plasma levels of HDL-C and triglycerides were measured at 7 examinations during 20 years of follow-up. In European Americans, rs328 (Ser447Stop), rs326, and rs13702 were significantly associated with cross-sectional interindividual variations in triglycerides and HDL-C and with their longitudinal changes over time. The minor alleles in rs326, rs328, and rs13702 that predispose an individual to lower triglycerides and higher HDL-C levels at young adulthood further slowed down the trajectory increase in triglycerides and decrease in HDL-C during 20 years of follow-up. In African-Americans, these 3 SNPs were significantly associated with triglycerides, but only rs326 and rs13702 were associated with HDL-C. rs328 showed a stronger association in European Americans than in African-Americans, and adjustment for this did not remove all of the associations for the other SNPs. Longitudinal changes in either trait did not differ significantly by SNP genotypes in African-Americans. Aging interacts with LPL gene variants to influence the longitudinal lipid variations.

There are some papers that reveal the importance of genetic screening for LPL gene mutations in a population at risk to develop hypertriglyceridemia. Some of these mutants, e.g. T-39C and T-93G, are located in the promoter region. The transcriptional activity of the -39 mutant promoter was less than 15% of wild-type, and that of the -93 mutant promoter was less than 50% of wild-type, as determined by transfection studies in a human macrophage-like cell line. Some other mutants are amino acidic changes, such as Asn291Ser, Asp9Asn and Ser447Ter. An association was demonstrated between the Asn291Ser substitution and decreased HDL cholesterol [148]. Familial combined hyperlipidemia (FCHL) patients carrying this mutation showed decreased HDL cholesterol and increased triglyceride levels compared to non-carriers. Presence of the D9N mutation was associated with hypertriglyceridemia and

reduced plasma high-density lipoprotein cholesterol concentrations. LPL-Asp9Asn carriers had higher diastolic blood pressure than non-carriers.

Cerebrovascular Risk and Hypertension

There is substantial evidence to suggest that blood pressure (BP) is an inherited trait. The introduction of gene technologies in the late 1980s generated a sharp phase of over-inflated prospects for polygenic traits such as hypertension. Not unexpectedly, the identification of the responsible loci in human populations has nevertheless proved to be a considerable challenge. Common variants of the RAS (renin-angiotensin system) genes, including those of *ACE* (angiotensin-converting enzyme) and *AGT* (angiotensinogen) were some of the first shown to be associated with BP. Presently, ACE and AGT are the only gene variants with functional relevance, where linkage studies showing relationships with hypertension have been reproduced in some studies and where large population-based and prospective studies have demonstrated these genes to be predictors of hypertension or BP. Nevertheless, a lack of reproducibility in other linkage and association studies has generated skepticism that only a concerted effort to attempt to explain will rectify. Without these explanations, it is unlikely that this knowledge will translate into the clinical arena. Angiotensinogen (*AGT*) gene polymorphisms have been linked to increased risk of hypertension, but the data remain controversial [118].

The genetic and environmental factors associated with the development of arterial hypertension are highly informative markers of the risk for developing cerebrovascular pathologies. 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.

The endothelial function and hypertension panel deals with the study of genes involved in cell migration and their contribution to the development of the atherosclerotic plaque as a trigger of stroke (**Table 4**).

Table 4. Polymorphisms related with cerebrovascular risk, endothelial function and hypertension

Gene Symbol	Gene Name	Locus	dbSNP	Polymorphism
ACE	Angiotensin I converting enzyme	17q23.3	rs4332	c.496-66T>C
AGT	Angiotensinogen	1q42.2	rs4762	c.620C>T; p.Thr207Met
			rs699	c.803T>C; p.Met268Thr
NOS3	Nitric oxide synthase 3	7q36.1	rs1799983	c.894G>T

ACE: Angiotensin I converting enzyme; *AGT*: Angiotensinogen; *NOS3*: Nitric oxide synthase 3.

***ACE*-Angiotensin I Converting Enzyme**

Angiotensin I converting enzyme is a dipeptidyl carboxypeptidase that plays an important role in regulating blood pressure and electrolyte balance [118]. Hydrolyses angiotensin I to angiotensin II, that is 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 [149,150], which predisposes to the suffering of other cardiovascular diseases.Several studies described that there was a significant association between *ACE* polymorphisms and brain lacunar infarction, intracranial hemorrhage and ischemic stroke [20,151-154], although other investigators could not detect the association [155-157].

Genetic variants of *ACE* are suspected risk factors in cardiovascular disease, but the alleles responsible for the variations remain unidentified. Johnson et al. [158] searched for regulatory polymorphisms, and allelic angiotensin I-converting enzyme (ACE) mRNA expression was measured in 65 heart tissues, followed by genotype scanning of the ACE locus. Marked allelic expression imbalance (AEI) detected in five African-American subjects was associated SNPs rs7213516, rs7214530, and rs4290, residing in conserved regions 2-3 kb upstream of ACE. Moreover, each of the SNPs affected transcription in reporter gene assays. SNPs rs4290 and rs7213516 were tested for associations with adverse cardiovascular outcomes in hypertensive patients with coronary disease (International Verapamil SR Trandolapril Study Genetic Substudy [INVEST-GENES]). Both SNPs were associated with adverse cardiovascular outcomes, largely attributable to nonfatal myocardial infarction in African Americans, showing an odds ratio of 6.16 for rs7213516. The high allele frequency in African Americans (16%) compared to Hispanics (4%) and Caucasians (< 1%) suggests that these alleles contribute to variation between populations in cardiovascular risk and treatment outcomes. The polymorphisms of angiotensinogen (*AGT*) and angiotensin-converting enzyme (*ACE*) genes

have been linked to increased risk of essential hypertension in multiple populations, but results have been inconsistent. Ji et al. [159] evaluated the associations of these polymorphisms with essential hypertension through a meta-analysis of the association studies within the Han Chinese population. They reviewed the two most commonly investigated polymorphisms, *AGT*M235T* and*ACE*I/D*, and provided summary estimates regarding their associations with essential hypertension. PubMed and China Biological Medicine Database were searched, and a total of 71 studies (31 studies for *AGT*M235T* and 40 studies for *ACE*I/D*) comprising 10547 essential hypertension patients and 9217 controls from 23 provinces and special districts in China were finally included in the study. Statistically significant associations with essential hypertension were identified for the TT genotype *of AGT*M235T* polymorphism and the DD genotype of *ACE*I/D* polymorphism. Under dominant, recessive, and additive genetic models, positive associations were also found. The heterogeneity existed among the studies, whereas the publication bias did not exist in both *AGT* analysis and *ACE* analysis. The meta-analysis suggests that *AGT*M235T* and *ACE*I/D* modulate the risk of essential hypertension in the Han Chinese population. There has been an increase in research into the association between *ACE* gene deletion polymorphism and cardiovascular disease, with conflicting results. To evaluate whether the DD genotype could also be associated with a higher prevalence of hypertension in healthy subjects over 6 years of follow-up, Di Pasquale et al. [160] conducted a long-term study on 684 healthy volunteers (aged 25-55 years), normotensive and free of cardiovascular diseases, with acceptable echocardiographic window. All subjects had to have a normal electrocardiogram (ECG) and echocardiogram (ECHO) at entry, and underwent a complete physical examination, 12-lead ECG and ECHO, and venous blood samples were drawn for DNA analysis and cholesterol. All subjects had a clinical evaluation each year for the 6-year duration of the study. All 684 subjects completed 6 years of follow-up. Three genetically distinct groups were identified. The ACE-DD group had 42 hypertensive subjects, 5 heart failure (HF) subjects and 6 subjects with acute coronary syndromes (ACS). There was no association between family history, smoking habit, hypercholesterolemia and events. The ACE-ID group had 16 hypertensive subjects and 3 subjects with ACS. The ACE-II group had 2 hypertensive subjects and 1 HF subject. The incidence of hypertension and cardiovascular events was significantly higher in the ACE-DD group than in the ACE-ID and ACE-II groups. The higher incidence of hypertension was observed in the older age groups (36-45 and 46-55 years) with ACE-DD and ACE-ID genotypes. These data suggest that the ACE-DD polymorphism is associated with a higher incidence of hypertension in baseline healthy subjects, irrespective of other risk factors. The higher incidence of hypertension was apparent predominantly in the older age groups.

AGT-Angiotensinogen

As a part of the Renin-Angiotensin system, the angiotensinogen precursor is expressed in the liver and is cleaved by renin in response to lowered blood pressure [118]. The resulting product, angiotensin I, is then cleaved by angiotensin converting enzyme (ACE) to generate the physiologically active enzyme angiotensin II. AGT is involved in maintaining blood pressure and in the pathogenesis of essential hypertension and preeclampsia [161,162].

To review the most commonly investigated polymorphisms at the *AGT* locus (other than M235T) and to provide summary estimates regarding their association with essential hypertension, while addressing heterogeneity as well as publication biases, data on 26818 subjects from 46 studies for the four most-studied *AGT* variants (T174M in exon 2 and 3 promoter variants: A-6G, A-20C, and G-217A) were meta-analyzed [163]. Statistically significant associations with hypertension were identified for the T174M and G-217A polymorphisms. A dual but consistent effect was observed for the -20C allele, which was associated with a decreased risk of hypertension in populations of mixed and European ancestries, but with a 24% increase in the odds of hypertension in Asian subjects. No association was detected of the A-6G variant with hypertension. Current studies support the notion that single variants at *AGT* might modulate the risk of hypertension. Intervention studies have indicated an interaction between the blood pressure response to a low-sodium or a low-fat and high-fruit and -vegetable diet and the angiotensinogen gene (*AGT*) polymorphisms G-6A and M235T. To investigate whether this interaction is also present in a large free-living population, urinary sodium and potassium as biomarkers of intake, and blood pressure, were measured in 11384 men and women aged 45-79 years participating in the Norfolk arm of the European Prospective Investigation of Nutrition and Cancer (EPIC). Highly significant associations between sodium and blood pressure were shown for all genotypes, but the regression coefficient for systolic blood pressure associated with each unit of sodium for each of the MT and TT genotypes was approximately double that for the MM homozygotes. Differences were evident at high exposures to sodium but not at low exposures. There were no significant associations between blood pressure and dietary or urinary potassium. This large cross-sectional study supports public health recommendations to reduce salt consumption in the population as a whole, and confirms intervention trial data showing the greatest response to intervention in persons with the AA and TT genotype in the *AGT* G-6A and M235T polymorphisms. Genotype effects in populations at low exposure to sodium are not likely to be seen. T174M (rs4762) showed complete linkage disequilibrium with M235T (rs699). T174M showed no correlation with any of the 4 clinical entities included in the study (essential hypertension, left ventricular hypertrophy, ischemic heart

disease, and myocardial infarction), but the T235 allele occurred more frequently in the essential hypertension group and less frequently in the group of myocardial infarction survivors. The frequency of T235 homozygotes was 70%, with 28% for T235 heterozygotes and only 2% for M235 homozygotes, the corresponding figures were 12%, 46%, and 42% in Caucasians.

Angiotensinogen and its cleaved forms angiotensin II and I, are important regulators of blood pressure. The gene for angiotensinogen (*AGT*) carries two common polymorphisms, T207M and M268T (previously described as T174M and M235T). To investigate the role of haplotypes formed by these polymorphisms for angiotensinogen levels, blood pressure, coronary artery disease (CAD), myocardial infarction (MI), and AGT genotypes and haplotypes were examined in 2575 patients with angiographically documented CAD and 731 individuals in whom CAD had been ruled out by angiography [164]. Three haplotypes, designated as Hap1 (T207, M268), Hap2 (T207, T268) and Hap3 (M207, T268), accounted for over 99% of alleles. The *AGT* Hap2 haplotype was significantly associated with angiotensinogen levels, one additional Hap2 allele accounted for an approx. 8% increase in angiotensinogen. This association was stronger than that of either single polymorphism. *AGT* genotypes or haplotypes were not related to hypertension, CAD or MI. A common haplotype of the angiotensinogen gene is linked to angiotensinogen levels but has no major impact on blood pressure, hypertension, or cardiovascular risk. The association of renin C-4063T and angiotensinogen (*AGT*) T174M, M235T and A-6G polymorphisms with ischemic stroke of atherosclerotic etiology was investigated in 329 Tunisian patients with stroke, and 444 controls [165]. *AGT*235T* and *AGT*-6G* allele and *AGT*235TT*, *AGT*-6AG* and *AGT*-6GG* genotype frequencies were higher in patients. Linkage disequilibrium (LD) was noted for *AGT*174T* with *AGT* 235M* and *AGT*-6A* in patients, while *AGT*235M* was in LD with *AGT*-6A* in controls and *AGT*235T* was in LD with *AGT*-6G* in both groups. The *AGT*174T/235T/-6A* and *AGT*174T/235M/-6G* haplotypes were positively and negatively associated with stroke, respectively. Multivariate regression analysis identified *AGT*174T/235M/-6A*, *AGT*174T/235T/-6G*, *AGT*174T/235T/-6A* and *AGT*174M/235T/-6A* haplotypes to be significantly associated with an increased risk of stroke. Renin-angiotensin-aldosterone system polymorphisms influence the risk of atherosclerotic stroke in Tunisians [165].

*AGT*polymorphisms are associated with susceptibility to essential hypertension [17], and can cause renal tubular dysgenesis, a severe disorder of renal tubular development [166] and non-familial structural atrial fibrillation [167].

NOS3-Nitric Oxide Synthase 3

This gene encodes one of the three isoforms of the nitric oxide synthase, the endothelial (eNOS) nitric oxide synthase [118]. The enzyme eNOS synthesizes nitric oxide from L-arginine. Nitric oxide acts as a biologic mediator in several processes, including neurotransmission and antimicrobial and anti-tumoral activities and blood pressure regulation.*NOS3* polymorphisms are described as a risk factor for endothelial dysfunction and hypertension [19], Alzheimer's disease [168,169], ischemic stroke [170], coronary spams and myocardial infarction [171].

NO-endothelium-dependent vasodilation is a mechanism that may affect blood pressure response and endothelial NO synthase (*eNOS* or *NOS3*) gene is a good candidate for the regulation of exercise blood pressure. Rankinen et al. [19] investigated the associations between theNOS3*Glu298Asp(894G>T) polymorphism and endurance training-induced changes in resting and submaximal exercise blood pressure in 471 white subjects of the HERITAGE Family Study. Both systolic and diastolic blood pressure at 50W decreased in response to the training program, whereas resting blood pressure remained unchanged. The decrease in diastolic blood pressure at 50W was greater (P=0.0005, adjusted for age, gender, baseline body mass index, and baseline diastolic blood pressure at 50 W) in the Glu298Glu homozygotes (4.4 [SEM 0.4] mm Hg, n=187) than in the heterozygotes (3.1 [0.4] mm Hg, n=213) and the Asp298Asp homozygotes (1.3 [0.7] mm Hg, n=71). The genotype accounted for 2.3% of the variance in diastolic blood pressure at 50W training response. Both the Glu298 homozygotes and the heterozygotes had a greater (P=0.013) training-induced reduction in rate-pressure product at 50W than the Asp298 homozygotes. These data suggest that polymorphism in *NOS3* gene locus is associated with the endurance training-induced decreases in submaximal exercise diastolic blood pressure and rate-pressure product in sedentary normotensive white subjects.

Molecular epidemiologic studies have presented contradictory results concerning a potential role of *NOS3*894G>T* polymorphism in Alzheimer's disease [168,169]. To define a possible association of this polymorphism with late onset AD in an Iranian population, a case-control study was conducted, including a clinically well-defined group of 100 AD patients and 100 age-matched controls. A significantly increased number of individuals with the *NOS3*894GG* genotype was observed in AD patients compared with controls.

Berger et al. [170] performed 2 large case-control studies involving 1901 hospitalized stroke patients and 1747 regional population controls and found that Glu298Asp was significantly associated with ischemic stroke independent of age, gender, hypertension, diabetes, and hypercholesterolemia.

Cerebrovascular Risk and Inflammation

Atherosclerosis can be considered to be a form of chronic inflammation resulting from interaction between modified lipoproteins, monocyte-derived macrophages, T cells, and the normal cellular elements of the arterial wall [10,24]. This inflammatory process can ultimately lead to the development of complex lesions, or plaques that appear in the arterial lumen. Plaque rupture results in the acute clinical complications of myocardial infarction and stroke [172].

Pro-inflammatory cytokines participate in the induction of ischemic stroke. Several studies showed increased concentration of the pro-inflammatory cytokines interleukin-1 (IL1) and interleukin-6 (IL6), as well as tumor necrosis factor TNF-alpha in blood and cerebrovascular fluid during ischemic stroke [173-175]. Polymorphisms in the 5' flanking region of these genes (promoter region) (**Table 5**) may alter the levels of expression and thus the concentration of this cytokines in the brain damaged regions [22,23,27-30].

Table 5. Polymorphisms related with cerebrovascular risk, immune response and inflammation

Gene Symbol	Gene Name	Locus	dbSNP	Polymorphism
IL1B	Interleukin 1 beta	2q13	rs1143634	c.3954T>C
IL6	Interleukin 6	7p15.3	rs1800795	c.-174G>C
			rs1800796	c.-573G>C
IL6R	Interleukin 6 receptor	1q21.3	rs8192284	c.1510A>C
TNF	Tumor necrosis factor	6p21.33	rs1800629	c.-308G>A

IL1B: Interleukin 1 beta; *IL6*: Interleukin 6; *IL6R*: Interleukin 6 receptor; *KCNJ11*: Potassium channel, inwardly rectifying subfamily J, member 11; *LEP*: Leptin; *LEPR*: Leptin receptor; *LIPC*: Lipase, hepatic; *LPL:* Lipoprotein lipase; *TNF*: Tumor necrosis factor.

IL1B-Interleukin 1 Beta

IL-1β is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE) [118]. IL-1βis an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. Interleukin-1 gene cluster polymorphisms have been related with increased risk of hypochlorhydria induced by Helicobacter pylori and gastric cancer [176], inflammatory bowel disease [177], Alzheimer's disease [178], and Parkinson's disease [179], as well as myocardial infarction and ischemic stroke [23].

Iacoviello et al. [23] found that patients carrying the *IL1B*512TT* genotype showed a decreased risk of myocardial infarction (OR, 0.36, 95% CI, 0.20-0.64) and stroke (OR, 0.32, 95% CI, 0.13-0.81) after adjustment for conventional risk factors.Mononuclear cells from volunteers carrying the T allele showed a decreased release of IL1and a decreased expression of tissue factor after stimulation with lipopolysaccharide compared with CC homozygotes.

Rios et al. [180] investigated the association of *IL1B* and *IL6* gene polymorphisms and angiographically assessed coronary artery disease (CAD) in African- and Caucasian-Brazilians. The authors analyzed the *IL1B*-511C>T* and *IL6*-174G>C* polymorphisms in 667 patients (253 African-Brazilians and 414 Caucasian-Brazilians) who underwent coronary angiography. Patients with a coronary obstructive lesion presented a higher frequency of the *IL1B*-511CC* genotype (30.4%) compared to lesion-free individuals (16.5%) in African- but not in Caucasian-Brazilians. No significant genotype frequency difference was identified for the *IL6*-174G>C* polymorphism in either ethnic groups. However, after correction for other CAD risk factors using multivariate logistic regression, both the *IL1B*-511CC* and the *IL6*-174GG* genotypes were considered independent CAD risk predictors in African-Brazilians. The *IL1B*-511C>T* and *IL6*-174G>C* polymorphisms were associated with CAD risk in African-Brazilians and no association was detected among Caucasian-Brazilians.

IL6-Interleukin 6

The cytokine encoded by the *IL6* gene functions in inflammation and the maturation of B cells [118]. IL-6 has been shown to be an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections. The protein is primarily produced at sites of acute and chronic inflammation, where it is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6-receptor alpha. This cytokine is implicated in a wide variety of inflammation-associated disorders, including diabetes mellitus, systemic juvenile rheumatoid arthritis, coronary artery disease, Intracranial hemorrhage, systemic lupus erythematosus, and others [181-186].

Interleukin-6 (IL6) is a pleiotropic cytokine involved in the regulation of the acute phase reaction, immune responses, and hematopoiesis.A polymorphism in the 5' flanking region of the *IL6* gene alters the transcriptional response to stimuli such as endotoxin and interleukin-1. This *IL6*-174G>C*polymorphism has been found to be associated to

different plasma IL6 levels in healthy volunteers [182]. Patients *IL6*-174CC* homozygotes showed significantly lower platelet count than carriers of the *IL6*-174G* allele, despite similar age, sex, body mass index and proportion of smokers [185].

It has been found that carriers of the *IL6*-174G* allele, which is associated with increased secretion of IL-6, have increased levels of plasma triglycerides, VLDL and free fatty acids, as well as lower levels of HDL-cholesterol. On the other hand, a strong association between the *IL6*-174CC* genotype and lacunar infarction has been described [29].

Multivariable logistic regression analysis with adjustment for conventional risk factors revealed that the *IL6*-573G>C* polymorphism was significantly (P<0.001) associated with both atherothrombotic cerebral infarction and intracerebral hemorrhage [33].

IL6R - Interleukin 6 Receptor

This gene encodes a subunit of the interleukin 6 (IL6) receptor complex. The IL6 receptor is a protein complex consisting of this protein and interleukin 6 signal transducer (IL6ST/GP130/IL6-beta), a receptor subunit also shared by many other cytokines [118]. Disregulated production of IL6 and this receptor are implicated in the pathogenesis of many diseases, such as multiple myeloma, autoimmune diseases and prostate cancer [187-189].

The *IL6R*1510A>C* polymorphism (rs8192284) is significantly associated with circulating levels of IL6SR. The *IL6R*1510C*variant has an incidence of 35% in Europeans and only 4% in Africans, accounting for differences in the concentration of circulating IL6SR, that is formed by cleavage of IL6R from the cell membrane [190].

In an Alzheimer's case-control study in Chinese population, Wang et al. [191] screened the *IL6R* promoter and the proteolytic cleavage site of IL-6R.*The IL6R*-530T* allele located in a putative regulatory region and the *IL6R*+48867C* allele at the splice site may elevate the risk of Alzheimer's disease.

TNF-Tumor Necrosis Factor

Tumor necrosis factor (TNF) is a multifunctional proinflammatory cytokine secreted predominantly by macrophages that isinvolved inregulation of lipid metabolism, coagulation, insulin resistance, and endothelial function [118,192,193].

The *TNF-308G>A* polymorphism(rs1800629) in the promoter region of the gene is associated with reduced circulating levels of TNF-alpha [194], and this variability may be related with a variety of diseases, including autoimmune diseases, insulin resistance, and cancer [195-197].

It is not clear what can be considered a risk variant, because the findings published are contradictory. On the one hand, the *TNF*-308AA*homozygoteshas been associated with increased levels of cortisol in saliva and obesity [30]. An association has also been described between *TNF*-308G* variant in homozygosis and an increased risk of migraine, probably due to the effect of this polymorphism on cerebral blood flow [27].

Cerebrovascular Risk and Thrombosis

Although advanced atherosclerotic lesions can lead to ischemic symptoms as a result of the progressive narrowing of the vessel lumen, acute cardiovascular events that result in myocardial infarction and stroke are generally thought to result from plaque rupture and thrombosis.

Patients with atrial fibrillation have a 5-fold increased risk of thromboembolic stroke, probably attributable to activation of blood coagulation [198].

Variations in coagulation factor II or prothrombin (*F2*), coagulation factor V Leiden (*F5*), and methylenetetrahydrofolate reductase (*MTHFR*), are especially important, increasing atherothrombotic risk (**Table 6**).

Table 6. Polymorphisms related with cerebrovascular risk and thrombosis

Gene Symbol	Gene Name	Locus	dbSNP	Polymorphism
F2	Coagulation factor II, thrombin	11p11.2	rs1799963	c.20210G>A
F5	Coagulation factor V	1q24.2	rs6025	c.1691G>A
HDAC9	Histone deacetylase 9	7p21.1	rs11984041	c.3162-3711C>T
MTHFR	Methylenetetrahydrofolate reductase (NAD(P)H)	1p36.22	rs1801133 rs1801131	c.665C>T; p.Ala222Val c.1286A>C; p.Glu429Ala

F2: Coagulation factor II, thrombin; *F5*: Coagulation factor V; *HDAC9*: Histone deacetylase 9; *MTHFR*: Methylenetetrahydrofolate reductase (NAD(P)H).

F2-Coagulation factor II, Thrombin

Coagulation factor II is proteolytically cleaved to form thrombin in the first step of the coagulation cascade, which ultimately results in the stemming of blood loss. Thrombin also plays a role in maintaining vascular integrity during development and postnatal life. Mutations in *F2* lead to various forms of thrombosis and dysprothrombinemia [118]. Thrombin, which cleaves bonds after arginine and lysine, converts fibrinogen to fibrin and activates factors V, VII, VIII, XIII, and, in complex with thrombomodulin, protein C.

The *F2*20210G>A* polymorphism (rs1799963) is found in 3% of the population of southern Europe. This alteration is associated with increased plasma levels of prothrombin. People who carry one copy of this mutation (20210A allele) are 6 times more likely to suffer a thrombosis. Pregnant women or those treated with contraceptives have a 16.3 times greater risk of thrombosis if they are carriers of the mutation [35].

Martinelli et al. [199] founded that the 20210G-A mutation in the prothrombin gene is associated with 'idiopathic' cerebral vein thrombosis.The presence of both the prothrombin gene mutation and oral contraceptive use raised further the risk of cerebral vein thrombosis. Cerebral vein thrombosis is a frightening event due to the severity of the clinical manifestations and the high mortality rate, estimated to be 5 to 30%. Clinically, cerebral vein thrombosis presents with a wide range of symptoms, including headache, focal deficits (motor or sensory), dysphasia, seizures, and impaired consciousness.

F5-Coagulation Factor V

This gene encodes Factor V Leiden, one of the factors involved in blood clotting. Factor V function is inactivated by protein C, which is one of the most important anticoagulant mechanisms. Thrombin, when bound to thrombomodulin on the endothelial surface, activates protein C and this in turn, inactivates factors V and VIII. The G1691A polymorphism (rs6025, Arg506Gln) in *F5* has a high prevalence in Caucasians, between 5 and 10% [118]. The presence of the 1691A mutation prevents inactivation of factor V by protein C, resulting in a state of hypercoagulability and increased thrombotic risk. Studies suggest an increase from 50 to 100 times the risk of venous thrombosis for homozygous carriers of the 506Q allele and 5 to 10 times for heterozygous carriers of R506Q [35].

Factor V is the plasma cofactor for the prothrombinase complex that activates prothrombin to thrombin. Congenital factor V deficiency is a bleeding disorder associated with mild to severe hemorrhagic symptoms and prevalence in the general population of 1 in a million in the homozygous form [200]. Patients with FV deficiency and clinically significant manifestations show very low or immeasurable plasma FV levels and are usually homozygous or compound heterozygous for mutations located in the *F5* gene [201]. Heterozygous carriers have approximately half-normal levels of FV and are usually asymptomatic. More than 60 mutations associated with FV deficiency and more than 700 polymorphisms that do not have a clinical phenotype have now been identified [118]. More than two thirds of these are null mutations, with the remaining being missense mutations.

Gain-of-function variants of genes encoding coagulation factor V (*F5*1691G>A*) and prothrombin (*F2*20210G>A*) cause hypercoagulability and are established risk factors for venous thrombosis. Manucci et al. [202] developed a meta-analysis of 66155 cases and 91307 controls and found that both polymorphisms are associated with a moderately increased risk of coronary artery disease (CAD). In 1880 patients with myocardial infarct (1680 men and 210 women) and an equal number of controls, the minor *F5*1691A*allele (2.6% frequency in cases and 1.7% in controls) was associated with an increased risk of myocardial infarct, the association remaining significant after adjustment for traditional risk factors.

Allele and genotype frequencies of three SNPs in the factor V gene leading to nonsynonymous changes (M385T in exon 8, and R485K and R506Q (Leiden mutation) in exon 10) were studied in 133 Caucasian women with pre-eclampsia and 112 healthy controls [203]. Haplotype frequencies were estimated using an expectation-maximization algorithm. Comparison of single-point allele and genotype distributions of SNPs in exons 8 and 10 of the factor V gene revealed statistically significant differences in R485K allele and genotype frequencies between the patients and the control subjects. The A allele of SNP R485K was over-represented among the patients (12%) vs the control subjects (4%), at an odds ratio (OR) of 2.8 for combined A genotypes (GA+AA vs GG). Allele and genotype differences between the patients and control subjects as regards M385T and Leiden mutation were not significant. In haplotype estimation analysis, there was a significantly elevated frequency of haplotype T-A-G encoding the M385-K485-R506 variant in the pre-eclamptic group vs the control group, at an OR of 2.6. The T-A-G haplotype was more frequent among the patient group than in the control group, and genetic variations in the factor V gene other than the Leiden mutation may play a role in disease susceptibility.

Ridker et al. [204] found that the R506Q mutation of the F5 gene was present in 25.8% of men over the age of 60 in whom primary venous thrombosis developed. There was no increased risk for secondary venous thrombosis. The presence of the mutation was not associated with an increased risk of myocardial infarction or stroke. In a follow-up study, of 77 study participants who had a first idiopathic venous thromboembolism, Ridker et al. [205] found that factor V Leiden was associated with a 4- to 5-fold increased risk of recurrent thrombosis. The data raised the

possibility that patients with idiopathic venous thromboembolism and factor V Leiden may require more prolonged anticoagulation to prevent recurrent disease compared to those without the mutation.

HDAC9-Histone Deacetylase 9

Histones play a critical role in transcriptional regulation, cell cycle progression, and developmental events. Histone acetylation/deacetylation alters chromosome structure and affects transcription factor access to DNA. The protein encoded by *HDAC9* gene has sequence homology to members of the histone deacetylase family. HDAC9 may play a role in hematopoiesis. A GWAS of the International Stroke Genetics Consortium (ISGC) identified a novel association for thepolymorphism rs11984041within the histone deacetylase 9 (*HDAC9*) gene on chromosome 7p21.1 which was associated with large vessel stroke in a further 735 cases and 28583 controls ($P=1.87\times10^{-11}$, OR, 1.42, 95% CI, 1.28-1.57) [104].

HDAC9 is ubiquitously expressed, with high levels of expression in cardiac tissue, muscle and brain. Although known as histone deacetylases, these proteins also act on other substrates and lead to both upregulation and downregulation of genes. To date no associations have been reported between rs11984041 or correlated SNPs and hypertension, hyperlipidaemia, or diabetes from large-scale GWAS of these risk factors [104].

MTHFR-Methylenetetrahydrofolate Reductase (NAD(P)H)

This gene encodes for Methylenetetrahydrofolate reductase, which catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for remethylation of homocysteine to methionine[118]. The 677C>T polymorphism (rs1801133, A222V) gives rise to a protein with reduced enzymatic activity and increased thermolability when the 222V variant is present. *MTHFR*677TT* individuals have high plasma homocysteine levels and have a risk of premature cardiovascular disease up to three times higher than the rest. Another mutation also related to a reduction in enzyme activity is A1298C (rs1801131, E429A), but this reduction in activity does not appear to be related to increased plasma homocysteine levels or lower concentrations of plasma folate as is the with 677T homozygotes. An increased intake of folate (folic acid 0,8 mg) reduces the risk of ischemic heart disease by 16% and that of stroke by 24% [37].

In a comprehensive meta-analysis of 22 case-control studies including 3387 white adult patients, Casas et al. [34] found a statistically significant association between ischemic stroke and the 677C>T substitution. Kelly et al. [206] performed a meta-analysis to determine the risk for ischemic stroke associated with hyperhomocysteinemia and the *MTHFR*677C>T* polymorphism. The data support an association between mild to moderate hyperhomocysteinemia and ischemic stroke. The *MTHFR*677TT* genotype may have a small influence in determining the susceptibility to ischemic stroke.

From studies of the 677C>T mutation in cardiovascular patients and controls, Kluijtmans et al. [207] found that homozygosity for this frequent mutation in the *MTHFR* gene is associated with a 3-fold increase in risk for premature cardiovascular disease. Klerk et al. [208] performed a meta-analysis of the risk of coronary heart disease related to the 677C>T polymorphism. They reported that individuals with the 677TT genotype have a significantly higher risk of coronary heart disease, particularly in the setting of low folate status. These results supported the hypothesis that impaired folate metabolism, resulting in high homocysteine levels, is causally related to increased risk of coronary heart disease. Schwartz et al. [209] concluded that this polymorphism was not a risk factor for myocardial infarction in their population. Schwartz et al. studied allele frequencies of the *MTHFR*677C>T* polymorphism in 69 non-Hispanic white female survivors of myocardial infarction and 338 controls. They found a similar distribution of alleles in both groups.

Pharmacogenetics of Cardiovascular Drugs

Pharmacogenetics offers the opportunity to greatly improve treatment through its personalization, avoiding problems such as high-risk interactions, adverse reactions or therapeutic inefficacy. This is a step towards eliminating the current trial-and-error method of drug prescription, where patients are subjected to different doses of drugs and/or different therapeutic options. The information provided by pharmacogenetic analysis is valuable, as a single genetic analysis will provide information on our metabolism of drugs that will be valid throughout our lifetime.

Bearing in mind the patient's genomic profile, the physician will choose from the list of drugs metabolized by each of the enzymes analyzed (**Table 7**). If the patient has a genetic variation (Ultra-rapid Metabolizer or Poor Metabolizer) in any of the genes analyzed, this means that this enzyme does not function "normally", and therefore, the drugs it metabolizes will be processed abnormally (**Figure 2**).

In cardio and cerebrovascular treatments, variability in efficacy and serious adverse effects continue to plague therapy. There are many sources of variability in response to drug therapy, such as noncompliance and unrecognized drug interactions, but translating pharmacogenomic discoveries to individual patients and populations is a challenge for genomic and personalized medicine.

Now we will review the different findings in the field of pharmacogenetics to improve treatments, as well as major pharmacogenes coding for enzymes, receptors and transporters involved in ADME processes (**Table 8**), such as absorption, distribution, metabolism and excretion of drugs, used in cardiovascular therapy: antithrombotic agents,

antihypertensives, antiarrhythmics, beta-blockers, and lipid modifying agents.

ABCB1-ATP-binding Cassette, Sub-family B (MDR/TAP), Member 1

The *ABCB1* gene encodes for P-glycoprotein (P-gp), considered the responsible for the multidrug resistance (MDR) phenotype. P-gp is expressed in various human tissues, such as the liver, kidney, pancreas, and the blood-brain barrier, although it is of particular interest to observe that P-gp is functionally expressed in the enterocytes surrounding the epithelium of the intestinal tract, where it plays an important role, in conjunction with metabolic processes, in the intestine's function as a barrier to medicines and xenobiotics in general. In the case of drugs, P-gp may determine the bioavailability of the same, independently of their chemical nature [118].

Table 7. Cardiovascular system drugs with relevant pharmacogenetic information

Antihemorrhagics	
Other systemic hemostatics	
Eltrombopag	*CYP1A2 - UGT1A1*
Antithrombotic agents	
Direct factor Xa inhibitors	
Apixaban	*CYP1A2*
Direct thrombin inhibitors	
Argatroban	*CYP3A4 - CYP3A5*
Platelet aggregation inhibitors	
Acetylsalicylic acid	*CYP2C9 - CYP3A4 - CYP3A5 - G6PD - UGT1A1*
Cilostazol	*CYP2D6 - CYP2C19 - CYP3A4 - CYP3A5 - CYP1A2*
Clopidogrel	*CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2 - ABCB1*
Prasugrel	*CYP2D6 - CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5*
Ticagrelor	*CYP3A4 - CYP3A5*
Ticlopidine	*CYP2C19 - CYP3A4 - CYP3A5*
Treprostinil	*CYP2C9*
Vitamin K antagonists	
Acenocoumarol	*CYP2C9 - CYP1A2 - VKORC1*
Phenprocoumon	*CYP2C9 - VKORC1*
Warfarin	*CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2 - UGT1A1 - VKORC1*
Agents acting on the renin-angiotensin system	
ACE inhibitors	
Captopril	*CYP2D6*
Enalapril	*CYP3A4 - CYP3A5*
Lisinopril	*CYP3A4 - CYP3A5*
Temocapril	*SLCO1B1*
Angiotensin II antagonists	
Candesartan	*CYP2C9*
Irbesartan	*CYP2C9 - CYP3A4 - CYP3A5*

Losartan	*CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2 - UGT1A1 - ABCB1*
Olmesartan	*CYP2C9 - SLCO1B1*
Valsartan	*CYP2C9 - SLCO1B1*
Renin inhibitors	
Aliskiren	*CYP3A4 - CYP3A5*
Antihypertensives	
Imidazoline receptor agonists	
Clonidine	*CYP1A2*
Methyldopa	*CYP3A4 - CYP3A5*
Alpha-adrenoreceptor antagonists	
Doxazosin	*CYP2D6 - CYP2C19 - CYP3A4 - CYP3A5*
Hydrazinophthalazine derivatives	
Hydralazine	*CYP2C9 - CYP3A4 - CYP3A5 - NAT2 - SLCO1B1*
Other antihypertensives	
Ambrisentan	*CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5*
Bosentan	*CYP2C9 - CYP3A4 - CYP3A5 - SLCO1B1*
Sitaxentan	*CYP2C9 - CYP3A4 - CYP3A5*
Beta blocking agents	
Alpha and beta blocking agents	
Carvedilol	*CYP2D6 - CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2 - UGT1A1*
Beta blocking agents non-selective	
Pindolol	*CYP2D6*
Propranolol	*CYP2D6 - CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2*
Sotalol	*CYP2D6 - CYP2C19*
Timolol	*CYP2D6 - CYP2C19*
Beta blocking agents selective	
Betaxolol	*CYP2D6 - CYP1A2*
Bisoprolol	*CYP2D6 - CYP3A4 - CYP3A5*
Celiprolol	*ABCB1*
Metoprolol	*CYP2D6 - CYP2C19*
Nebivolol	*CYP2D6*
Talinolol	*ABCB1*
Calcium channel blockers	
Benzothiazeoine derivatives	
Diltiazem	*CYP2D6 - CYP2C9 - CYP3A4 - CYP3A5 - ABCB1*

Phenylalkylamine derivatives	
Verapamil	*CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2 - ABCB1*
Dihydropyridine derivatives	
Amlodipine	*CYP3A4 - CYP3A5*
Felodipine	*CYP3A4 - CYP3A5*
Isradipine	*CYP3A4 - CYP3A5*
Lacidipine	*CYP3A4 - CYP3A5*
Nicardipine	*CYP2D6 - CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2*
Nifedipine	*CYP2D6 - CYP3A4 - CYP3A5*
Nimodipine	*CYP3A4 - CYP3A5*
Nisoldipine	*CYP3A4 - CYP3A5*
Nitrendipine	*CYP3A4 - CYP3A5*
Antiarrhythmics	
Antiarrhythmics class Ia	
Disopyramide	*CYP3A4 - CYP3A5*
Procainamide	*CYP2D6 - NAT2*
Quinidine	*CYP2C9 - CYP3A4 - CYP3A5 - ABCB1*
Antiarrhythmics class Ib	
Lidocaine	*CYP2D6 - CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2*
Mexiletine	*CYP2D6 - CYP3A4 - CYP3A5 - CYP1A2*
Antiarrhythmics class Ic	
Flecainide	*CYP2D6 - CYP1A2*
Propaphenone	*CYP2D6 - CYP3A4 - CYP3A5 - UGT1A1*
Antiarrhythmics class III	
Amiodarone	*CYP2D6 - CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2*
Dofetilide	*CYP3A4 - CYP3A5*
Dronedarone	*CYP3A4 - CYP3A5*
Other antiarrhythmics	
Moricizine	*CYP3A4 - CYP3A5*
Vernakalant	*CYP2D6*
Cardiac glycosides	
Digitalis glycosides	
Digoxin	*CYP3A4 - CYP3A5 - ABCB1*
Other cardiac preparations	
Ibuprofen	*CYP2D6 - CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5 - UGT1A1*
Indometacin	*CYP2D6 - CYP2C19 - CYP2C9 - CYP3A4 - CYP3A5 - UGT1A1*

Ivabradine	*CYP3A4 - CYP3A5*
Ranolazine	*CYP2D6 - CYP3A4 - CYP3A5*
Isosorbide	*CYP3A4 - CYP3A5*
Diuretics	
Sulfonamides (high-ceiling diuretics)	
Bumetanide	*NAT2*
Furosemide	*NAT2*
Piretanide	*NAT2*
Torasemide	*CYP2C9 - NAT2*
Sulfonamides (low-ceiling diuretics)	
Chlortalidone	*NAT2*
Clofenamide	*NAT2*
Clopamide	*NAT2*
Clorexolone	*NAT2*
Fenquizone	*NAT2*
Indapamide	*CYP3A4 - CYP3A5 - NAT2*
Mefruside	*NAT2*
Meticrane	*NAT2*
Metolazone	*NAT2*
Xipamide	*NAT2*
Vasopressin antagonists	
Conivaptan	*CYP3A4 - CYP3A5*
Tolvaptan	*CYP3A4 - CYP3A5*
Aldosterone antagonists	
Eplerenone	*CYP3A4 - CYP3A5*
Other potassium-sparing agents	
Triamterene	*CYP2D6 - CYP3A4 - CYP3A5 - CYP1A2*
Lipid modifying agents	
Fibrates	
Bezafibrate	*CYP3A4 - CYP3A5 - UGT1A1*
Ciprofibrate	*UGT1A1*
Clofibrate	*UGT1A1*
Fenofibrate	*CYP3A4 - CYP3A5 - UGT1A1*
Gemfibrozil	*CYP3A4 - CYP3A5 - UGT1A1*
HMG CoA reductase inhibitors	
Atorvastatin	*CYP2C9 - CYP3A4 - CYP3A5 - UGT1A1 - ABCB1 - SLCO1B1*

Cerivastatin	*SLCO1B1*
Fluvastatin	*CYP2D6 - CYP2C9 - CYP3A4 - CYP3A5*
Lovastatin	*CYP3A4 - CYP3A5 - ABCB1 - SLCO1B1*
Pitavastatin	*CYP2C9 - CYP3A4 - CYP3A5 - SLCO1B1*
Pravastatin	*CYP3A4 - CYP3A5 - ABCB1 - SLCO1B1*
Rosuvastatin	*CYP2C9 - CYP3A4 - CYP3A5 - SLCO1B1*
Simvastatin	*CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2 - ABCB1*
Other lipid modifying agents	
Ezetimibe	*UGT1A1*
Peripheral vasodilators	
Ifenprodil	*CYP2C19*
Vasoprotectives	
Corticosteroids	
Betamethasone	*ABCB1*
Dexamethasone	*CYP3A4 - CYP3A5 - CYP1A2 - ABCB1*
Fluocinonide	*ABCB1*
Fluocortolone	*ABCB1*
Fluorometholone	*ABCB1*
Hydrocortisone	*ABCB1*
Prednisolone	*CYP3A4 - CYP3A5 - ABCB1*
Triamcinolone	*ABCB1*
Local anesthesics	
Lidocaine	*CYP2D6 - CYP2C9 - CYP3A4 - CYP3A5 - CYP1A2*
Bioflavonoids	
Diosmin	*UGT1A1*
Hidrosmin	*UGT1A1*
Monoxerutin	*UGT1A1*
Rutoside	*UGT1A1*
Troxerutin	*UGT1A1*

The three most widely studied polymorphisms of *ABCB1* are 1236C>T, Gly412Gly (rs1128503), 2677G>T/A, Ala893Thr/Ser (rs2032582) and 3435C>T, Ile1145Ile (rs1045642), which define the haplotypes most frequently associated with multidrug resistance: *ABCB1*1* (CGC) (high resistance), with a frequency of 36.84% in Europeans, and *ABCB1*2* (TTT) (low resistance), with a frequency of 40.89% in Europeans.The TTT haplotype (*ABCB1*2*) is associated with reduced methylation of the gene promoter, which gives rise to a reduced expression of *ABCB1*, while the CGC haplotype (*ABCB1*1*) is associated with hypermethylation of the promoter and over-expression of *ABCB1*.

Clopidogrel absorption and thereby active metabolite formation are diminished by P-gp-mediated efflux and are influenced by the *ABCB1*3435C>T* genotype. Pharmacogenetic determinants of the response of patients to clopidogrel contribute to variability in the biologic

antiplatelet activity of the drug. The effect of these determinants on clinical outcomes after an acute myocardial infarction is unknown. Patients with two variant alleles of *ABCB1* (3435TT) had a higher rate of cardiovascular events at 1 year than those with the *ABCB1* wild-type genotype (3435CC) [210].

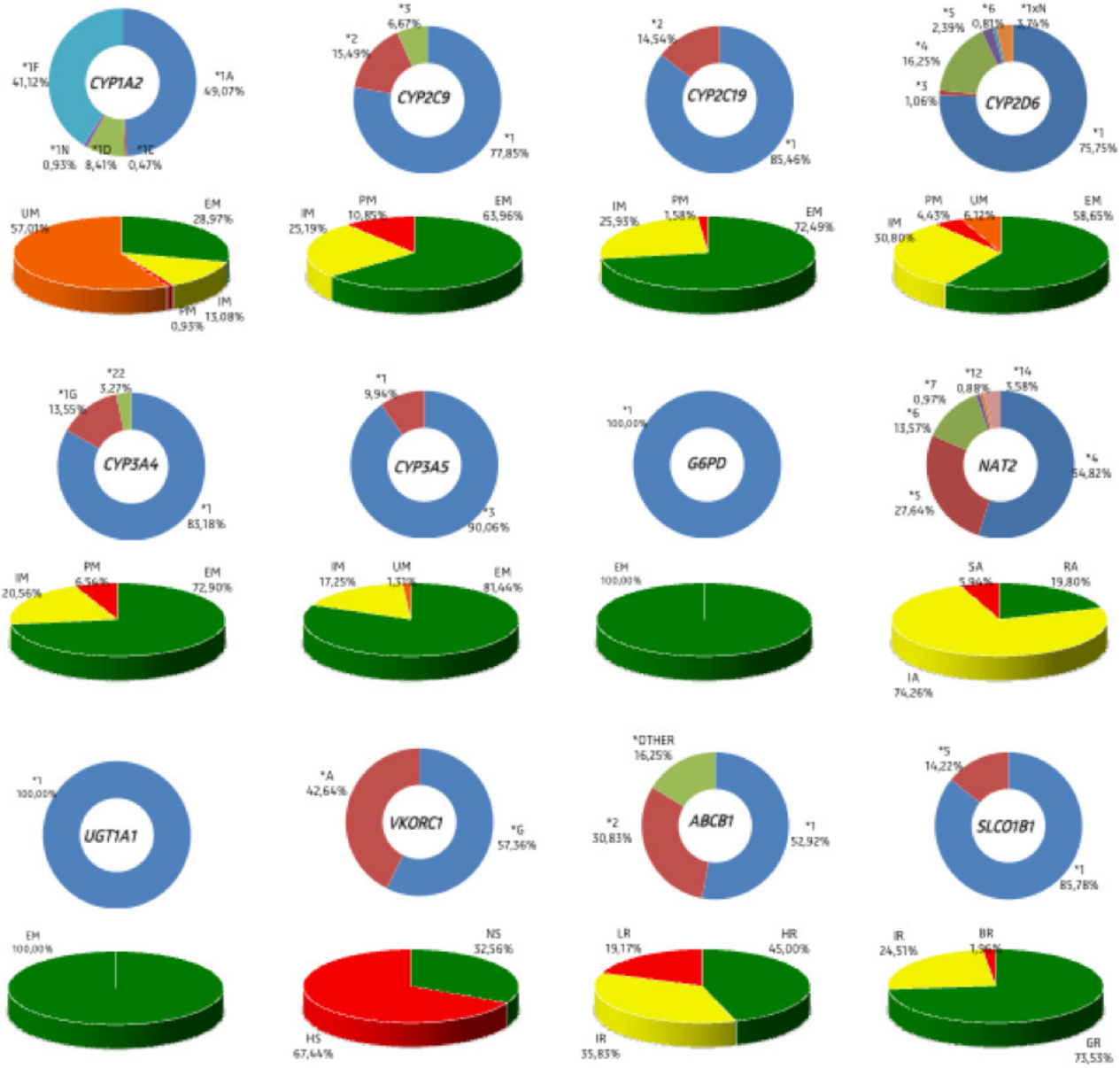

Figure 2. Impact of pharmacogenetic polymorphisms in Spanish population (N=2834)

ABCB1 polymorphisms, particularly 3435C>T, may affect drug transport and efficacy. Mega et al. [211] assessed the effect of this polymorphism by itself and alongside variants in CYP2C19 on cardiovascular outcomes in patients treated with clopidogrel or prasugrel in TRITON-TIMI 38. Mega et al.[211] genotyped *ABCB1* in 2932 patients with acute coronary syndromes undergoing percutaneous intervention who were treated with clopidogrel (n=1471) or prasugrel (n=1461) in the TRITON-TIMI 38 trial. They evaluated the association between *ABCB1*3435C>T* and rates of the primary efficacy endpoint (cardiovascular death, myocardial infarction, or stroke) until 15 months, and then assessed the combined effect of *ABCB1*3435C>T* genotype and reduced-function alleles of *CYP2C19*. In patients treated with clopidogrel, *ABCB1*3435C>T* genotype was significantly associated with the risk of cardiovascular death, myocardial infarction, or stroke. TT homozygotes had a 72% increased risk of the primary endpoint compared with CT/CC individuals. *ABCB1*3435C>T* and *CYP2C19* genotypes were significant, independent predictors of the

primary endpoint, and 681 (47%) of the 1454 genotyped patients taking clopidogrel who were either *CYP2C19* reduced-function allele carriers, *ABCB1*3435 TT* homozygotes or both, were at increased risk of the primary endpoint.

Table 8. Polymorphisms related with pharmacogenetics of cardiovascular drugs

Gene Symbol	Gene Name	Locus	dbSNP	Polymorphism
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	7q21.1	rs1128503	c.1236C>T; pGly412Gly
			rs2032582	c.2677G>T/A
			rs1045642	c.3435C>T
ADRB1	Adrenoceptor beta 1	10q25.3	rs1801252	c.145A>G; p.Ser49Gly
			rs1801253	c.1165G>C; p.Gly389Arg
CACNB2	Calcium voltage-gated channel auxiliary subunit beta 2	10p12	rs2357928	c.-558G>A
CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide 2	5q24.1	rs2069514	g.28338G>A; *1C
			rs35694136	c.-1635delT; *1D
			rs762551	c.9-154C>A; *1F
CYP2C19	Cytochrome P450, family 2, subfamily C, polypeptide 19	10q24	rs4244285	c.681G>A, P227P; *2
			rs12248560	c.-806C>T; *17
CYP2C9	Cytochrome P450, family 2, subfamily C, polypeptide 9	10q24	rs1799853	c.430C>T, p.Arg144Cys; *2
			rs1057910	c.1075A>C,p. Ile359Leu; *3
CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6	22q13.2	rs35742686	c.775delA; p.Arg259Glyfs; *3
			rs3892097	c.506-1G>A; *4
			dup/del	*1xN (Dup); *5 (Del)
			rs5030655	c.454delT; p.Trp152Glyfs; *6
			rs28371725	c.985+39G>A; 41
CYP3A4	Cytochrome P450, family 3 subfamily A, polypeptide 4	7q21.1	rs2242480	c.1026+12G>A; *1G
			rs35599367	c.522-191C>T; *22
CYP3A5	Cytochrome P450, family 3 subfamily A, polypeptide 5	7q21.1	rs776746	c.219-237G>A; *3
G6PD	Glucose-6-phosphate dehydrogenase	Xq28	rs1050828	c.202G>T; p.Val68Met
			rs5030868	c.563C>A; Ser188Phe
NAT2	N-acetyltransferase 2 (arylamine N-acetyltransferase)	8p22	rs1801280	c.341T>C; p.Ile114Thr; *5
			rs1799930	c.590G>A; p.Arg197Gln; *6
			rs1799931	c.857G>A; p.Gly286Glu; *7
			rs1799929	c.481C>T; p.Leu161Leu; *11
			rs1208	c.803G>A; p.Arg268Lys; *12
			rs1041983	c.282C>T; p.Tyr94Tyr; *13

			rs1801279	g.191G>A; *14
SLCO1B1	Solute carrier organic anion transporter family, member 1B1	12p	rs4149056	c.521T>C; p.Val174Ala; *5
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1	2q37	rs4148323	c.211G>A; *6
VKORC1	Vitamin K epoxide reductase complex, subunit 1	16p11.2	rs9923231	c.-1639G>A

ABCB1: ATP-binding cassette, sub-family B (MDR/TAP), member 1; *ADRB1*: Adrenoceptor beta 1; CACNB2: Calcium voltage-gated channel auxiliary subunit beta 2; *CYP1A2*: Cytochrome P450, family 1, subfamily A, polypeptide 2; *CYP2C19*: Cytochrome P450, family 2, subfamily C, polypeptide 19; *CYP2C9*: Cytochrome P450, family 2, subfamily C, polypeptide 9; *CYP2D6*: Cytochrome P450, family 2, subfamily D, polypeptide 6; *CYP3A4*: Cytochrome P450, family 3 subfamily A, polypeptide 4; *CYP3A5*: Cytochrome P450, family 3 subfamily A, polypeptide 5; *G6PD*: Glucose-6-phosphate dehydrogenase; *NAT2*: N-acetyltransferase 2 (arylamine N-acetyltransferase); *SLCO1B1*: Solute carrier organic anion transporter family, member 1B1; *UGT1A1*: UDP glucuronosyltransferase 1 family, polypeptide A1; *VKORC1*: Vitamin K epoxide reductase complex, subunit 1.

Digoxin is a cardiotonic glycoside obtained mainly from *Digitalis lanata*, it consists of three sugars and the aglycone digoxigenin. Digoxin has positive inotropic and negative chronotropic activity. It is used to control ventricular rate in atrial fibrillation and in the management of congestive heart failure with atrial fibrillation. Its use in congestive heart failure and sinus rhythm is less certain. The margin between toxic and therapeutic doses is small. No influence is noted of *ABCB1*2677G>A/T* and *ABCB1*3435C>T* polymorphisms on digoxin concentration. Although some studies [212-214] have shown that digoxin pharmacokinetics might be affected by *ABCB1* genetic polymorphism, those modest changes are probably clinically irrelevant, and digoxin dose adjustment should include P-gp inhibitor co-administration rather than *ABCB1* genotyping. Carriers of two T alleles for the C3435T polymorphism in exon 26 of *ABCB1* tend to have a lower apparent volume of distribution than carriers of a C allele. *ABCB1*1236C>T*, 2677G>T, and 3435C>T variants and the associated TTT haplotype were associated with higher digoxin serum concentrations in a cohort of elderly European digoxin users in the general population. 2677T (Ser893) has been associated with increased efflux of digoxin in vitro. In vitro expression of *ABCB1* encoding Ala893 (*ABCB1*1*) or a site-directed Ser893 mutation (*ABCB1*2*) indicated enhanced efflux of digoxin by cells expressing the ABCB1*Ser893 variant. 3435TT genotypes have higher plasma levels and lower intestinal expression.

Takara et al. [213] examined ABCB1 (P-glycoprotein)-mediated interaction between digoxin and 29 antihypertensive drugs. Most of the Ca^{2+} channel blockers used markedly inhibited basal-to-apical transport and increased apical-to-basal transport. Exceptions were diltiazem, nifedipine and nitrendipine, which hardly showed inhibitory effects on transcellular transport of [3H] digoxin. Alpha-blocker doxazosin and beta-blocker carvedilol also inhibited transcellular transport of [3H] digoxin, but none of the angiotensin converting enzyme inhibitors and AT1 angiotensin II receptor antagonists used were active.

In the PLATO trial of ticagrelor vs clopidogrel for treatment of acute coronary syndromes, ticagrelor reduced the composite outcome of cardiovascular death, myocardial infarction, and stroke, but increased events of major bleeding related to non-coronary artery bypass graft (CABG). *CYP2C19* and *ABCB1* genotypes are known to influence the effects of clopidogrel. Wallentin et al. [215] investigated the effects of these genotypes on outcomes between and within treatment groups. DNA samples obtained from patients in the PLATO trial were genotyped for *CYP2C19* loss-of-function alleles (*2, *3, *4, *5, *6, *7, and *8), the *CYP2C19* gain-of-function allele *17, and the *ABCB1* SNP 5C>T. For the *CYP2C19* genotype, patients were stratified by the presence or absence of any loss-of-function allele, and for the *ABCB1* genotype, patients were stratified by predicted gene expression (high, intermediate, or low). The primary efficacy endpoint was the composite of cardiovascular death, myocardial infarction, or stroke after up to 12 months' treatment with ticagrelor or clopidogrel. 10285 patients provided samples for genetic analysis. The primary outcome occurred less often with ticagrelor vs clopidogrel, irrespective of *CYP2C19* genotype: 8.6% vs 11.2% in patients with any loss-of-function allele, and 8.8% vs 10.0% in those with no loss-of-function allele. For the *ABCB1* genotype, event rates for the primary outcome were also consistently lower in ticagrelor than in the clopidogrel group for all genotype groups. In the clopidogrel group, the event rate at 30 days was higher in patients with than in those without loss-of-function *CYP2C19* alleles (5.7% vs 3.8%), leading to earlier separation of event rates between treatment groups in patients with loss-of-function alleles. Patients on clopidogrel who had any gain-of-function *CYP2C19* allele had a higher frequency of major bleeding (11.9%) than did those with no gain-of-function or loss-of-function alleles (9.5%), but interaction between treatment and genotype groups was not significant for any type of major bleeding. Ticagrelor is a more efficacious treatment for acute coronary syndromes than is clopidogrel,

irrespective of *CYP2C19* and *ABCB1* polymorphisms. Use of ticagrelor instead of clopidogrel eliminates the need for the currently recommended genetic testing before dual antiplatelet treatment.

ADRB1-Adrenoceptor Beta 1

The adrenergic receptors (subtypes alpha 1, alpha 2, beta 1, and beta 2) are a prototypic family of guanine nucleotide binding regulatory protein-coupled receptors that mediate the physiological effects of the hormone epinephrine and the neurotransmitter norepinephrine.

ADRB1 encodes the adrenoceptor beta 1, the primary target of beta-blocking agents [118]. Two specific polymorphisms in this gene, *ADRB1*145A>G* (Ser49Gly, rs1801252) and *ADRB1*1165G>C* (Gly389Arg, rs1801253) have been shown to correlate with hypertensionand myocardial infarction risk, and antihypertensive (atenolol) and beta-blocker (bisoprolol, metoprolol, timolol, verapamil) responses to these conditions [216].

The Ser49Gly polymorphism was associated with lower resting heart rate in hypertensive patients, independent of beta-blocker therapy [217]. In two studies, Ser49 homozygotes experienced a significantly greater blood pressure reduction than Gly carriers after treatment with metoprolol [217]. Haplotype analysis of the variants at codons 49 and 389 revealed that those with the Ser49Gly389/Gly49Arg389 (*ADRB1*H2/H3*) haplotype were virtually unresponsive to metoprolol, whereas the greatest response was observed in subjects with the *ADRB1*H1/H1* haplotype (Ser49Arg389/Ser49Arg389) (other combinations were intermediate) [218].

CACNB2-Calcium Voltage-Gated Channel Auxiliary Subunit Beta 2

CACNB2 encodes the beta 2 subunit of the L-type voltage-dependent calcium channel protein that is a member of the voltage-gated calcium channel superfamily. The beta 2 regulatory subunit control the cell surface expression of the alpha 1c subunit, the pore-forming subunit to which all calcium channel blockers bind.

The INVEST-GENES study showsa different genotype-dependent outcome in antihypertensive treatment with beta-blockers or calcium channel blockers. Rs2357928*GG patients randomized to calcium channel blockers were more likely to experience an adverse outcome than those randomized to bets-blockers treatment strategy, with adjusted hazard ratio (HR) of 2.35 (95% CI, 1.19-4.66, P=□0.014) [219].

CYP1A2-Cytochrome P450, Family 1, Subfamily A, Polypeptide 2

The cytochrome P450 1A2 acts on 5-10% of drugs in current clinical use. CYP1A2 is responsible for more than 95% of the primary metabolism of caffeine [220], and has been shown to be important in the metabolism of clozapine [221].

CYP1A2 activates several aromatic amines and thus is a key enzyme in chemical carcinogenesis. Several studies on the CYP1A2-dependent metabolism of caffeine or phenacetin have demonstrated that this enzyme is expressed in human livers at various levels amongst individuals, suggesting polymorphic control of enzyme activity [222].

CYP1A2 plays a major role in the metabolism of many commonly used cardiovascular drugs, including antithromboticagents (acenocoumarol, apixaban, clopidogrel, cilostazol, warfarin), antiarrhythmics (amiodarone, flecainide, lidocaine, mexiletine), betablockers (betaxolol, carvedilol, propranolol), and other relevant drugs as losartan, simvastatin and verapamil [118].

Clopidogrel is metabolically activated by several hepatic cytochrome P450 (CYP) isoenzymes, including CYP1A2. Cigarette smoking induces CYP1A2 and may, therefore, enhance the conversion of clopidogrel to its active metabolite.Clopidogrel therapy in smokers is associated with increased platelet inhibition and lower aggregation as compared with non-smokers. The mechanism of the smoking effect deserves further study and may be an important cause of response variability to clopidogrel therapy [223,224].Enhanced clopidogrel response in smokers, known as the smokers' paradox, is not universal but was observed only in *CYP1A2*-163A* allele carriers, suggesting a genotype-dependent effect of smoking on clopidogrel responsiveness [225].

Ishida et al. [226] studied the enzyme responsible for the stereoselective metabolism of carvedilol in the cells. The expression of *CYP1A1*and *CYP1A2* mRNA, but not *CYP2D6*, *CYP3A4*, and *CYP2C9* mRNA, was increased in beta-NF-treated Caco-2 cells, as compared with non-treated cells.

CYP2C19-Cytochrome P450, Family 2, Subfamily C, Polypeptide 19

CYP2C19 acts on 5-10% of drugs in current clinical use. About 2-6% of individuals of European origin, 15-20% of Japanese, and 10-20% of Africans have a slow acting, poor metabolizer form of this enzyme. However there is wide variability among populations. For example, the percentage of Polynesians who are poor metabolizers ranges from 38-79% depending on location. CYP2C19 is an important drug metabolizing enzyme that catalyzes the biotransformation of many other clinically useful drugs including antidepressants, barbiturates, proton pump inhibitors, antimalarial and antitumor drugs [118].

In cardiovascular therapy CYP2C19 is involved in the metabolism of antithrombotic agents (cilostazol, clopidogrel, prasugrel, ticlopidine, and warfarin), betablockers (carvedilol, metoprolol, propranolol, sotalol, timolol),

antihypertensives (ambrisentan, doxazosin), indometacin, losartan, verapamil and others.

Yoo et al. [227] investigated the influence of genetic polymorphisms in the *CYP3A5*, *CYP2C19* and *ABCB1* genes on the population pharmacokinetics of cilostazol in healthy subjects. The genetic polymorphisms of *CYP3A5* had a significant influence on the apparent oral clearance of cilostazol. When *CYP2C19* was evaluated, a significant difference was observed among the three genotypes (extensive metabolizers, intermediate metabolizers and poor metabolizers) for the apparent oral clearance. A combination of *CYP3A5* and *CYP2C19* genotypes was found to be associated with a significant difference in the apparent oral clearance. When including these genotypes, the interindividual variability of the apparent oral clearance was reduced from 34.1% in the base model to 27.3% in the final model. However, no significant differences between the *ABCB1* genotypes and cilostazol pharmacokinetic parameters were observed. *CYP3A5* and *CYP2C19* polymorphisms explain the substantial interindividual variability that occurs in the metabolism of cilostazol.

Several clinical studies have confirmed the effect of *CYP2C19* polymorphisms on the pharmacokinetics and/or pharmacodynamics of clopidogrel. Hulot et al. [228] determined whether frequent functional variants of genes coding for candidate CYP450 isoenzymes involved in clopidogrel metabolic activation (*CYP2C19*2*, *CYP2B6*5*, *CYP1A2*1F*, and *CYP3A5*3* variants) influence platelet responsiveness to clopidogrel. In healthy subjects, carriers of the *CYP2C19*1/*2*genotype had a reduced response to clopidogrel compared to the wild-type carriers during maintenance treatment at 75 mg daily. The *CYP2C19*2* allele was associated with higher platelet aggregability and residual platelet reactivity in high-risk vascular patients on dual antiplatelet treatment. Kim et al. [229] found that the AUC of clopidogrel for PMs of *CYP2C19* was 1.8-and 2.9-fold higher than that for heterozygous and homozygous EMs of *CYP2C19*, respectively. The Cmax of clopidogrel in PMs of *CYP2C19* was 1.8- and 4.7- fold higher than that of heterozygous and homozygous EMs, respectively. PMs of *CYP2C19* showed a significantly lower antiplatelet effect than EMs.

Prasugrel is a newly marketed antiplatelet drug with improved cardiac outcomes as compared with clopidogrel for acute coronary syndromes involving percutaneous coronary intervention (PCI). Analysis of a subset of the TRITON-TIMI 38 trial [230] demonstrated that *CYP2C19* reduced-function genotypes are associated with differential clinical responses to clopidogrel, but not prasugrel. An exploratory, secondary analysis was undertaken to estimate the clinical benefit of prasugrel over clopidogrel in subgroups defined by *CYP2C19* genotype, by integrating the published results of the genetic substudy and the overall TRITON-TIMI 38 trial. Individuals with a *CYP2C19* reduced-metabolizer genotype were estimated to have a substantial reduction in the risk of the composite primary outcome (cardiovascular death, myocardial infarction, or stroke) with prasugrel as compared with clopidogrel. For *CYP2C19* extensive metabolizers (~70% of the population), however, the composite outcome risks with prasugrel and clopidogrel were not substantially different. Integration of the TRITON-TIMI 38 data suggests that the *CYP2C19* genotype can discriminate between individuals who receive extensive benefit from using prasugrel instead of clopidogrel, and individuals with comparable clinical outcomes with prasugrel and clopidogrel.

CYP2C9-Cytochrome P450, Family 2, Subfamily C, Polypeptide 9

CYP2C9 acts on 15% of drugs in current clinical use. About 35% of Caucasians have a slow acting form of this enzyme. CYP2C9 is an important drug-metabolizing enzyme that catalyses the biotransformation of many other clinically useful drugs including angiotensin II blockers (candesartan, irbesartan, losartan, olmesartan, valsartan), antithrombotics (acenocoumarol, aspirine, clopidogrel, prasugrel, phenprocoumon, treprostinil, warfarin), antiarrhythmics (amiodarone, lidocaine, quinidine), antihypertensives (ambrisentan, bosentan, sitaxentan), statins (atorvastatin, fluvastatin, pitavastatin, rosuvastatin, simvastatin), non-steroidal anti-inflammatory drugs, alkylating anticancer pro-drugs, sulfonylureas and many others.

Of special interest are those drugs with narrow therapeutic window, such as S-warfarin, tolbutamide and phenytoin, where impairment in CYP2C9 metabolic activity might cause difficulties in dose adjustment as well as toxicity. Indications for testing include lack of therapeutic effect or difficulties with side effects to any of the drugs metabolized by CYP2C9.

Acenocoumarol, an analogue of warfarin, is a short-acting coumarin anticoagulant with a half-life of 8 h. The main metabolic route of racemic acenocoumarol is 6- and 7-monohydroxylation, while 8-hydroxylation is a minor pathway. All hydroxylated metabolites are further conjugated to their corresponding O-glucuronides and O-sulfates. The metabolic clearance of S-acenocoumarol is high with a short plasma t½ of 2 h, thus the pharmacological effect lies almost exclusively with the R-enantiomer, unless there is a decreased *CYP2C9* activity (for example, presence of a *CYP2C9*3* allele). Only *CYP2C9* hydroxylated S- and R-acenocoumarol at the 6-, 7-, and 8-position, R-acenocoumarol was also metabolized by *CYP1A2* (6-hydroxylation) and *CYP2C19* (6-, 7-, and 8-hydroxylation). There is increasing clinical evidence that the *CYP2C9*3* allele is related to a low-dose requirement for this drug, a higher frequency of over-anticoagulation and an unstable or delayed stable anticoagulant response, and even one copy of *CYP2C9*3* might profoundly reduce the oral drug clearance. Spreafico et al. [231] performed a prospective study during

the initial phase of acenocoumarol therapy, analyzing the effect of *CYP2C9* variant alleles and *VKORC1* haplotypes, single and in combination, in 220 Italians. *CYP2C9*3* was associated with a 25% dose reduction and an increased risk of over-anticoagulation (INR>6).

Candesartan is a long-acting, nonpeptide, and selective angiotensin receptor antagonist used in the treatment of hypertension and congestive heart failure. Candesartan is released from its ester racemic prodrug (candesartan cilexitil) by presystemic hydrolysis in the intestinal wall. Candesartan is primarily excreted as unchanged drug (75%) in the urine (33%) and feces (67%) with a smaller proportion (20-25%) inactivated via O-deethylation by hepatic CYP2C9 to an inactive metabolite (CV-15959).Since the contribution of CYP2C9 to the overall clearance of candesartan is moderate in vivo (~20-25%), it can be expected that polymorphisms of *CYP2C9* would produce a moderate effect on the clearance of candesartan. However, a deficient allele of *CYP2C9* could cause a significant effect on candesartan clearance (48% lower) and plasma levels (2.5-fold higher), and it appears that the contribution of CYP2C9 to the overall clearance of candesartan is close to 48% in some patients in vivo, probably due to reduced renal and biliary excretion of the parent drug which normally accounts for about 75% of a total dose [232].

Brandt et al. [233] determined the relationship between genetic variation in CYP450 isoenzymes and the pharmacokinetic/pharmacodynamic response to prasugrel and clopidogrel. In patients receiving clopidogrel treatment, carriers of *CYP2C9*2* or *3 variants had significantly lower AUC and Cmax values of clopidogrel active metabolite and reduced inhibition of platelet aggregation and poor response compared to those with the wild-type genotype. Twelve out of 16 subjects (75.0%) with the *CYP2C9*2/*2* or *3 allele were poor responders, while only 41.4% (24/58) of the patients without the *CYP2C9*2/*2* or *3 allele were poor responders.

Inhibitors of HMG-CoA reductase, also known as "statins", represent an important group of therapeutic agents for the treatment of hypercholesterolemia, a major risk factor for the development of coronary artery disease. Fluvastatin is 50-80% metabolized by CYP2C9 to 5-hydroxy-, 6-hydroxy-, and N-deisopropyl-fluvastatin.The *CYP2C9*3/*3* genotype was associated with 3-fold higher concentrations of the more active (+)-3R,5S-fluvastatin than the wild-type in healthy subjects, while individuals carrying *the CYP2C9*1/*3* and *2/*3 genotypes had intermediate concentrations. Pharmacokinetics of both enantiomers showed statistically significant differences according to the number of *CYP2C9*3* alleles [234].

Hallberg et al. [235] studied whether the *CYP2C9* genotype influences the blood pressure-decreasing response to antihypertensive treatment with irbesartan. Hypertensive patients with the *CYP2C9*1/*2* genotype treated with irbesartan showed a greater decrease in diastolic blood pressure than the patients with the wild-type genotype, with a trend for a reduction in systolic blood pressure. However, there was no correlation between the *CYP2C9* genotype and blood pressure response to atenolol, a drug not metabolized via *CYP2C9*. The *CYP2C9* genotype seems to predict the diastolic blood pressure response to irbesartan, but not to atenolol, in patients with essential hypertension.

Phenprocoumon is used for the prophylaxis and treatment of thromboembolic disorders.The AUC of phenprocoumon metabolites after oral intake of 12 mg racemic phenprocoumon was significantly lower in volunteers expressing the *CYP2C9*2* or *CYP2C9*3* allele. Increasing plasma AUC metabolic ratios in *CYP2C9*2* and *CYP2C9*3* variant allele carriers were found for each hydroxylation reaction and the *CYP2C9*3/*3* genotype corresponded to an about 10-fold higher metabolic ratio of S-7-hydroxylation relative to *CYP2C9*1/*1*. *CYP2C9* polymorphisms cause a markedly compromised S-7-hydroxylation of phenprocoumon [236].

CYP2C9 polymorphisms have been shown to have a significant impact on incidence of bleeding episodes in patients receiving warfarin therapy. Patients with *CYP2C9* variants were more likely to have difficulties in achieving target INR range at the time of induction of warfarin therapy, and to have increased risk of major bleeding complications [237]. In this study, 56% of patients receiving a low daily dose of warfarin (≤1.5 mg) had INR values greater than 4, compared to 17% in the control group receiving a wide range of dosages. Patients with *CYP2C9*2* or **3* alleles had a greater risk of bleeding. The relative bleeding risk for *CYP2C9*2* was 1.91 and for *CYP2C9*3* 1.77, while the relative risk was 2.26 for either variant [238]. The reason for the unstable INR values and increased bleeding risks in patients with variant CYP2C9 alleles is unclear, but may be associated with decreased hydroxylation of S-warfarin in vivo with increased levels of warfarin exposure, and thus over-anticoagulant activity is expected to occur in individuals carrying these mutant alleles.

CYP2D6-Cytochrome P450, Family 2, Subfamily D, Polypeptide 6

CYP2D6 acts on 25% of all prescription drugs. 7-14% of the population has a slow acting form of this enzyme and 7% a super-fast acting form.35% are carriers of a non-functional CYP2D6 allele, which especially elevates the risk of adverse drug reactions when these individuals are taking multiple drugs [118].

Drugs that CYP2D6 metabolizes include selective serotonin reuptake inhibitors (SSRI), tricyclic antidepressants (TCA), opiates, neuroleptics and a variety of toxic plant substances. Specific cardiovascular drugs are antiarrhythmics (amiodarone, flecainide, lidocaine, mexiletine, procainamide, propaphenone, vernakalant) and beta-blockers

(betaxolol, bisoprolol, carvedilol, metoprolol, nebivolol, pindolol, propranolol, sotalol, timolol). CYP2D6 is also responsible for activating the pro-drug codeine and other opioids into their active forms. The analgesic activity of these drugs is therefore reduced or absent in CYP2D6 poor metabolizers [118].

The association between *CYP2D6*4* and blood pressure or heart rate was examined in 1533 users of beta-blockers in the Rotterdam Study, a population-based cohort study [239]. In*CYP2D6 *4/*4* PMs, the adjusted heart rate in metoprolol users was 8.5 beats/min lower compared with *1/*1 extensive metabolizers (EMs), leading to an increased risk of bradycardia in PMs. The diastolic blood pressure in PMs was 5.4 mm Hg lower in users of beta-blockers metabolized by CYP2D6 and 4.8 mm Hg lower in metoprolol users compared with EMs. PMs are at increased risk of bradycardia. Patients with cardiovascular diseases are often treated by concurrent multiple drug therapy. It is therefore plausible that with an increasing number of drugs the risk of drug interactions increases. Such interactions can be either pharmacodynamic (and are due to the mechanism of the administered drugs) or they can be pharmacokinetic (resulting in a reduction or enhancement of drug elimination). Pharmacokinetic interactions can be either due to interactions at the level of drug metabolizing enzymes (most importantly CYP450 enzymes), or interactions at the level of drug transporter proteins (for example P-glycoprotein (ABCB1)). It is important to distinguish between both mechanisms since interactions at transporter proteins can be attributed to those drugs that are not enzymatically metabolized. Four beta-blockers are widely used in the therapy of cardiovascular diseases, namely atenolol, bisoprolol, metoprolol, and carvedilol. Among these beta-blockers, atenolol is mainly eliminated by renal excretion, bisoprolol is in part excreted as parent compound via the renal route (50%), the other 50% are hepatically metabolized, whereas metoprolol and carvedilol are metabolized by CYP2D6. Evidence is accumulating that carvedilol is a substrate for P-glycoprotein. For these four beta-blockers various pharmacodynamic and pharmacokinetic interactions have been demonstrated. Such interactions that result in altered pharmacokinetics are mainly observed with those beta-blockers that are excreted via metabolism (metoprolol and carvedilol).

Carvedilol is a beta-adrenoceptor antagonist used for treating chronic heart failure (CHF). For 40 Japanese patients evaluated in a clinical study, the *CYP2D6 *1*, *10, and *5 genotypes were determined using allele-specific primer PCR, and individual patients' oral clearance (CL/F) of both enantiomers were estimated by the empirical Bayes method. Individual CL/F values for carvedilol were significantly lower in Japanese CHF patients with the *CYP2D6 *1/*5*, *5/*10 and *10/*10 genotypes. Estimation of the population pharmacokinetic parameters and their covariates for each enantiomer in patients with CHF showed that the CL/F values for R- and S-carvedilol were dependent on body weight, alpha1-acid glycoprotein and *CYP2D6* genotype [240].

Statin therapy, although generally well tolerated, leads not infrequently to significant subjective and at times objective adverse effects, mainly of a muscular nature. The genetic background of these adverse effects is not clear and possibly side effects and lipid lowering efficacy may be linked. None of the assessed CYP450 polymorphisms appeared to be related to an increased incidence of adverse effects. The *CYP2D6 *1/*4* and *4/4* poor metabolizer (PM) status was associated with a higher efficacy of statins metabolized by this system and, in addition, the *APOE-2* genotype was, in this series, linked to increased HDL-C levels after therapy. Patients with statin-associated myopathy are not characterized by significantly different genotypes for the CYP450s responsible for statin metabolism. *CYP2D6 PM* status is associated to an increased efficacy of statins metabolized by this system [241].

Mexiletine is an antiarrhythmic agent pharmacologically similar to lidocaine. It may have some anticonvulsant properties. Mexiletine is used for the control of ventricular arrhythmias and for neuropathic pain from cancer or diabetes mellitus. It is metabolized mainly by CYP2D6 and, to a lesser extent, by CYP1A2. In vitro studies with human liver microsomes have shown that the oxidative conversion of mexiletine (MX) to its metabolites is catalyzed by CYP2D6 and is significantly impaired in microsomes with the CYP2D6*10/*10 genotype [242]. Clearance of MX in the *CYP2D6*5/*10* subjects was comparable to that in poor metabolizers. Carriers of the *CYP2D6*10* allele showed a decreased clearance of MX. Subjects with *CYP2D6*5/ *10* showed significantly increased plasma levels of MX, and homozygotes for *CYP2D6*10* also showed an increase, although to a lesser extent. Thus, the *CYP2D6*10* allele plays an important role in MX pharmacokinetics.

CYP3A4/5-Cytochrome P450, Family 3 Subfamily A, Polypeptides 4/5

Cytochrome P450 3A4 (CYP3A4) and their isoform CYP3A5 act on approximately half of drugs in clinical use. About 5% of individuals of European origin have a slow acting, intermediate metabolizer form of CYP3A4. Prevalence of CYP3A5 variants differs widely by ethnic origin. People of African ancestry have an increased prevalence of CYP3A5 Rapid (*1/*3) or Ultra Rapid (*1/*1) metabolizer status. CYP3A4 and CYP3A5 are closely related and may process many of the same drugs. Substrates include opioid pain medications, statins, chemotherapeutic drugs and combined oral contraceptives [118].

Aliskiren is a renin inhibitor used in the treatment of hypertension. Itraconazole, a strong CYP3A4 inhibitor, raises the peak plasma aliskiren concentration 5.8-fold (range 1.1- to 24.3-fold) and the area under the plasma

aliskiren concentration-time curve 6.5-fold (range 2.6- to 20.5-fold) but has no significant effect on aliskiren elimination half-life [243]. Itraconazole increases the amount of aliskiren excreted into the urine during 12 hours 8.0-fold and its renal clearance 1.2-fold. Plasma renin activity 24 hours after aliskiren intake is 68% lower during the itraconazole phase than during the placebo phase. Itraconazole markedly raises the plasma concentrations and enhances the renin-inhibiting effect of aliskiren. The interaction is probably mainly explained by inhibition of the P-glycoprotein-mediated efflux of aliskiren in the small intestine, with a minor contribution from inhibition of CYP3A4. Concomitant use of aliskiren and itraconazole is best avoided.

Amiodarone has been reported to be involved in a significant number of drug interactions. It is mainly metabolized by CYP3A4 and is a potent inhibitor of CYP1A2, 2C9, 2D6 and 3A4. In addition, amiodarone may interact with other drugs (such as digoxin) via the inhibition of the P-glycoprotein membrane transporter system.

Close correlations between amiodarone N-monodesethylase activities and the amounts of CYP3A4, and the rates of lidocaine N-monodesethylation were observed [244]. Lidocaine inhibited amiodarone N-monodesethylation competitively, inversely, amiodarone suppressed lidocaine N-monodesethylase activity in the same manner. The interaction between amiodarone and lidocaine may be explained by the inhibition of CYP3A4 by amiodarone and/or by its main metabolite DEA.

To predict the drug interactions of amiodarone and other drugs, the inhibitory effects and inactivation potential for human CYP enzymes by amiodarone and its N-dealkylated metabolite, desethylamiodarone, were examined [245]. Amiodarone weakly inhibited CYP2C9, CYP2D6, and CYP3A4-mediated activities with Ki values of 45.1-271.6 μM. Desethylamiodarone competitively inhibited the catalytic activities of CYP2D6 and noncompetitively inhibited CYP2A6, CYP2B6, and CYP3A4. The catalytic activities of CYP1A1, CYP1A2, CYP2C9, and CYP2C19 were inhibited by desethylamiodarone with mixed type. Amiodarone inactivated CYP3A4, while desethylamiodarone inactivated CYP1A1, CYP1A2, CYP2B6, and CYP2D6. The interactions between amiodarone and other drugs might occur via the inhibition of CYP activities by its N-dealkylated metabolite, desethylamiodarone, rather than by amiodarone itself. The inactivation of CYPs by desethylamiodarone as well as by amiodarone would also contribute to the drug interactions.

Ticagrelor is a platelet inhibitor used to reduce the risk of thrombotic cardiovascular events for patients with acute coronary syndrome. It is metabolized by CYP3A4, and to a lesser extent, CYP3A5. There is a FDA label for this drug, which suggests avoiding using ticagrelor with CYP3A inhibitors (such as atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin and voriconazole) and inducers (such as rifampin, dexamethasone, phenytoin, carbamazepine, and phenobarbital). Ticagrelor also inhibits ABCB1, so digoxin levels should be monitored. In vitro studies have shown no inhibitory effect on human CYP1A2, CYP2C19 and CYP2E1 activity. Finally, the PLATO trial showed that bleeding with ticagrelor was not significantly affected by CYP2C19 genotype [246]. Ticagrelor will result in higher serum concentrations of simvastatin and lovastatin because these drugs are metabolized by CYP3A4.

Clopidogrel and statins are frequently administered in patients with ischemic heart disease or other atherothrombotic manifestations and are effective in the prevention of cardiovascular disease. The thienopyridine clopidogrel is a pro-drug metabolized in the liver via the cytochrome P450 (CYP) 3A4 system to the active compound, which inhibits the P2Y(12) ADP platelet receptor. The assumption exists that the effect of clopidogrel in inhibiting platelet aggregation is attenuated by co-administration of lipophilic statins such as atorvastatin or simvastatin, which are metabolized by the CYP3A4 system to inactive substrates [247].

G6PD-Glucose-6-Phosphate Dehydrogenase

The *G6PD* gene encodes glucose-6-phosphate dehydrogenase. The protein is a cytosolic enzyme encoded by a housekeeping X-linked gene whose main function is to produce NADPH, a key electron donor in the defense against oxidizing agents and in reductive biosynthetic reactions. *G6PD* is remarkable for its genetic diversity. Many variants of *G6PD*, mostly produced from missense mutations, have been described with wide-ranging levels of enzyme activity and associated clinical symptoms. G6PD deficiency may cause neonatal jaundice, acute haemolysis, or severe chronic non-spherocytic haemolytic anaemia. Several transcript variants encoding different isoforms have been found for this gene. G6PD is in the hexose monophosphate pathway, the only NADPH-generation process in mature red cells, which lack the citric acid cycle. For this reason G6PD deficiency has adverse physiologic effects. It produces pentose sugars for nucleic acid synthesis. The G6PD variants have been divided into 5 classes according to the level of enzyme activity. These are: class 1: enzyme deficiency with chronic non-spherocytic haemolytic anaemia, class 2: severe enzyme deficiency (less than 10%), class 3: moderate to mild enzyme deficiency (10-60%), class 4: very mild or no enzyme deficiency (60%), class 5: increased enzyme activity [118].

NAT2-N-Acetyltransferase 2 (Arylamine N-Acetyltransferase)

N-Acetyltransferase 2 (NAT2) plays an important role in the detoxification and/or metabolic activation of certain therapeutic drugs, occupational chemicals and carcinogens.

The enzyme produced by *NAT2* acts on 1% of drugs in current clinical use including isoniazid, a common tuberculosis treatment, and numerous chemicals. Approximately 50% of people in the United States are slow acetylators and 40% intermediate acetylators [118].

Arylamine N-acetyltransferase 2is a polymorphic phase II enzyme responsible for slow or rapid acetylation of the antihypertensive hydrazine, the antiarrhythmic procainamide and different high- and low-ceiling diuretics belonging to sulfonamides. Various combinations of SNPs have been identified as *NAT2* alleles or haplotypes. Different combinations of these SNPs in the *NAT2* coding region result in proteins with altered stability, degradation, and/or kinetic characteristics. These effects of SNPs on NAT2 proteins are the basis for slow, intermediate, and rapid acetylator phenotypes. Individuals homozygous for rapid NAT2 acetylator alleles are deduced as rapid acetylators, individuals homozygous for slow acetylator NAT2 alleles are deduced as slow acetylators, and individuals possessing one rapid and one slow NAT2 allele are deduced as intermediate acetylators.Haplotype definition includes the seven most frequent single nucleotide polymorphisms (SNPs) of *NAT2* including 191G>A, 282C>T, 341T>C, 481C>T, 590G>A, 803A>G, and 857G>A. The *NAT2*4* allele encodes for a fully active enzyme and is traditionally considered the wild type (rapid acetylator) allele. The representative four common alleles (haplotypes) that possess signature nucleotide substitutions at positions 341, 590, 857, and 191 are designated *NAT2*5*, *NAT2*6* and*NAT2*7*, respectively, and several studies have shown that the members of these clusters are responsible for the slow acetylator phenotype. In addition to the *NAT*4* haplotype, variants *NAT2*11*, *NAT2*12*, *NAT2*13* and *NAT2*14* are responsible for the rapid acetylator phenotype.

Hydralazine is a vasodilator used to treat hypertension. Hydralazine is thought to be metabolized by two pathways, both of which involve acetylation. One is via direct acetylation, forming the metabolite 3-methyl-s-triazolo [3,4-a]-phthalazine (MTP), and 3-OH-MTP [248]. Another is via oxidation to form an unstable intermediate compound that is acetylated to form N-acetylhydrazinophthalazine (NAcHPZ). Acetylation status has been associated with PK parameters of hydralazine. After oral dose, rapid acetylators display lower hydralazine plasma concentrations and area under the concentration-time curve (but no real difference in drug half life) compared to slow acetylators [249]. MTP/hydralazine ratio can be used to divide a population into slow and rapid acetylators, with a lower and higher ratio, respectively [250]. In one study, patients with a slow acetylator genotype displayed significant reductions in blood pressure measurements at 24 hours before and after hydralazine, whereas significant effects were not observed in rapid or intermediate acetylators [251]. Three out of a total of four patients who presented hydralazine-induced adverse reactions had a slow acetylator genotype [251]. However, evidence for hydralazine dose adjustment based on acetylator status is not clear.

Okumura et al. [252] studied the genotypes of polymorphic N-acetyltransferase (NAT2) in 145 Japanese subjects. They found that the acetylation activity substantially varied interindividually (procainamide to N-acetylprocainamide) but this variability was considerably reduced after classification according to the genotype. The N-acetylprocainamide/procainamide ratio in urinary excretion was 0.60±0.17 for those with *NAT2*4/*4*, 0.37±0.06 for *NAT2*4/*6A*, 0.40±0.03 for *NAT2*4/*7B*, and 0.17 for *NAT2*6A/*7B*. The results indicated that the *NAT2* genotype correlates with acetylation of procainamide.

One detoxification pathway for sulfonamides is N-acetylation of the parent drug by N-acetyltransferases (NATs), leading to an inactive metabolite that is eliminated in the urine. Polymorphisms in the NAT2 gene that lead to a defective "slow" N-acetylation phenotype are well described, and slow NAT2 phenotype and genotypes have been reported to be overrepresented in patients with sulfonamide hypersensitivity [253]. However, since slow *NAT2* polymorphisms are found in about half of Caucasians and African Americans, these genotypes alone are not sufficient to lead to sulfonamide hypersensitivity in most patients.

SLCO1B1-Solute Carrier Organic Anion Transporter Family, Member 1B1

The solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) gene encodes for a membrane-bound sodium-independent organic anion transporter protein (OATP1B1) that is involved in active cellular influx of many endogenous and xenobiotic compounds. OATP1B1 mediates active transport of many endogenous substrates, such as bile acids, xenobiotic compounds, and a wide panel of pharmaceutical compounds [118].

OATP1B1-dependent transport is an important step in mediating drug hepatic clearance. We would like to highlight one class of drugs, the HMG-CoA reductase inhibitors (statins) because statins are widely prescribed for cardiovascular disease (CVD) risk reduction. OATP1B1 transport is particularly important for hepatic accessibility of pravastatin, as this compound is too hydrophilic to gain significant hepatocellular entry through passive transport. OATP1B1-dependent transport could well be important for the acid (active) form of simvastatin, (and other statins less hydrophobic than pravastatin) as *SLCO1B1* variants were recently associated with simvastatin-induced myopathies, implying that OATP1B1 was involved with simvastatin transport [248]. Nine studies with 1360 cases and 3082 controls were included. Cases of statin-related myopathy were found to be significantly associated with the variant C allele, especially when statin-related myopathy was defined as an elevation of creatine kinase (CK) >10 times the upper

limit of normal (ULN) or rhabdomyolysis. When stratified by statin type, the association was significant in individuals receiving simvastatin, but not in those receiving atorvastatin. The available evidence suggests that *SLCO1B1* gene T521C polymorphism is associated with an increased risk of statin-related myopathy, especially in individuals receiving simvastatin. Thus, a genetic test before initiation of statins may be meaningful for personalizing the treatment [254].

In 2011 and updated in 2013, the FDA added warnings to the simvastatin product label to direct providers away from initiating at the 80 mg simvastatin dose. The FDA recommends against 80mg daily simvastatin dosage. In patients with the C allele at *SLCO1B1* rs4149056, there are modest increases in myopathy risk even at lower simvastatin doses (40mg daily), if optimal efficacy is not achieved with a lower dose, alternate agents should be considered [255].

UGT1A1-UDP Glucuronosyltransferase 1 Family, Polypeptide A1

The uridine diphosphate glucuronosyltransferase (UGT) enzymes are a superfamily of enzymes responsible for the glucuronidation of target substrates. The transfer of glucuronic acid renders xenobiotics and other endogenous compounds water soluble, allowing for their biliary or renal elimination. The UGT family is responsible for the glucuronidation of hundreds of compounds, including hormones, flavonoids and environmental mutagens.

One of the main functions of UGT1A1 is in the liver, where it is the only enzyme responsible for the metabolism of bilirubin, the hydrophobic breakdown product of heme catabolism. In general, UGT1A enzymes have considerable overlap in substrate specificities, however no other isozyme can substitute for the bilirubin glucuronidation activity of UGT1A1 [118].

*UGT1A1*28* occurs with a frequency of 26-31% in Caucasians, and 42-56% in African Americans, and only 9-16% in Asian populations. *UGT1A1*6* has allele frequencies in Japanese, Korean and Chinese populations of 13%, 23% and 23%, respectively [118].

There is also evidence suggesting that the UGT1A1*28 allele may offer protection from cardiovascular disease. Bilirubin is a known antioxidant, and is thought to be capable of preventing plaque formation leading to atherosclerosis [256]. Since the *28 allele impairs transcription of the UGT1A1 gene, it is associated with significantly increased bilirubin concentrations, and therefore could be an important biomarker for predicting those at decreased risk of cardiovascular disease [257].

Both the *28 and *6 alleles have been well studied in regard to pharmaceutical toxicities. In particular, both alleles have shown associations with the development of irinotecan toxicities. Besides irinotecan, UGT1A1 is also responsible for the glucuronidation of drugs such as raloxifene and etoposide, and some associations have been reported between the *28 allele and pharmacokinetic and pharmacodynamic parameters for these drugs. Additionally, the development of hyperbilirubinemia during treatment with inhibitors of UGT1A1, such as atazanavir and tranilast, has also been linked to the presence of the *28 allele.

VKORC1-Vitamin K Epoxide Reductase Complex, Subunit 1

The *VKORC1* gene encodes the vitamin K epoxide reductase (VKORC1) protein, which is a key enzyme in the Vitamin K cycle. VKORC1 is a 163 amino acid integral membrane protein associated with the endoplasmic reticulum, and VKORC1 mRNA is broadly expressed in many different tissues. VKORC1 is responsible for the conversion of Vitamin K-epoxide to Vitamin K, which is the rate-limiting step in the physiological process of Vitamin K recycling [118]. The availability of reduced Vitamin K is of particular importance for several coagulation factor proteins that require it as a cofactor, including Factor VII, Factor IX, and Factor X. VKORC1 is of therapeutic interest both for its role in contributing to high inter-patient variability in coumarin anticoagulant dose requirements and as a potential player in vitamin K-deficiency disorders [258].

Warfarin is an anticoagulant used as a prophylaxis and to treat venous thrombosis, pulmonary embolism, thromboembolic complications from atrial fibrillation and cardiac valve replacement, and to reduce the risk of stroke after a myocardial infarction. The FDA recommends genetic testing for *CYP2C9* and *VKORC1* variants prior to initiating treatment with warfarin [259].

CYP2C9 and *VKORC1* variation greatly affect the half-life of warfarin and time to a stable dose. The level of the enzyme is under genetic control according to the DNA sequence present in the control region of the gene. Inherited differences in *VKORC1* increase or decrease the amount of warfarin needed to inhibit the formation of the clotting factors. When the amount of warfarin exceeds what is needed, the risk of bleeding is increased. Indications for testing include lack of therapeutic effect or difficulties with side effects to warfarin.

The *VKORC1*-1639G>A* polymorphism is associated with lower dose requirements for warfarin in Caucasian and Asian patients [260]. Increased bleeding risk and lower initial warfarin dose requirements have been associated with the *CYP2C9*2* and *CYP2C9*3* alleles. Approximately 30% of the variance in warfarin dose could be attributed to genetic variation in *VKORC1*, and about 40% of dose variance could be explained taking into consideration both *VKORC1* and *CYP2C9* genetic polymorphisms [261]. Accounting for genetic variation in both *VKORC1* and *CYP2C9*, age, height, body weight, interacting drugs, and indication for warfarin therapy explained about 55% of the variability in warfarin dose.

The initial and maintenance dosing of warfarin must be individualized for each patient. The goal of warfarin therapy is to achieve an international normalized ratio (INR) in a target range for the condition being treated (most commonly 2-3). This involves selecting an initial starting dose, followed by regular testing of the INR so that the dose of warfarin can be adjusted until the appropriate daily maintenance dose is determined. In general, the duration of anticoagulant therapy varies by clinical indication and should be continued until the danger of thrombosis and embolism has passed.

The variants that are routinely tested for are *CYP2C9*2*, *CYP2C9*3*, and *VKORC1*-1639G>A*. These variants are used in the FDA table to guide therapy, and also in the International Warfarin Pharmacogenomics Consortium (IWPC) algorithm [262].

The pharmacogenetic algorithms available on the warfarindosing.org website [263] should be used whenever possible to determine the dose of warfarin required. Such algorithms have been derived from large studies across different ethnic populations, and they take into account both the genetic and non-genetic factors that influence the variability in warfarin response.

CONCLUSIONS

1. Cerebrovascular disorders and stroke are multifactorial and polygenic-related disorders that occur by the interaction of multiple environmental factors and sequence variations in different genes.
2. Atherosclerosis, the main pathological process leading to cerebrovascular disease, begins in youth or adulthood, remaining asymptomatic for 20 or 30 years until the onset of the disease.
3. Vascular genetic risk can be detected early and can affect healthy recommendations to prevent the development of cerebrovascular diseases.
4. Knowledge of genes involved in the development of cerebrovascular diseases enables us to make certain predictions regarding the risks, susceptibilities or resistance to developing them.
5. The ability to identify high-risk patients through genetic testing could make screening for treatable intermediate phenotypes more cost-effective.
6. Predictive genetic tests must integrate multigenic panels identifying variations in the DNA sequence related with development, prognosis and evolution of cerebrovascular disorders, representing a key tool in medical practice.
7. Pharmacogenetics offers the opportunity to greatly improve treatment through its personalization, avoiding problems such as high-risk interactions, adverse reactions or therapeutic inefficacy.
8. Pharmacogenetics is responsible for over 80% of the efficacy and safety of drugs, however, unawareness of the pharmacogenetic profile of the population and the lack of pharmacogenetic information in the leaflet of drugs cause over 50% of medical prescriptions to prove unsuitable.
9. The information provided by pharmacogenetic analysis is very valuable. A single genetic analysis provides information on response to drugs that will be valid throughout lifetime.

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