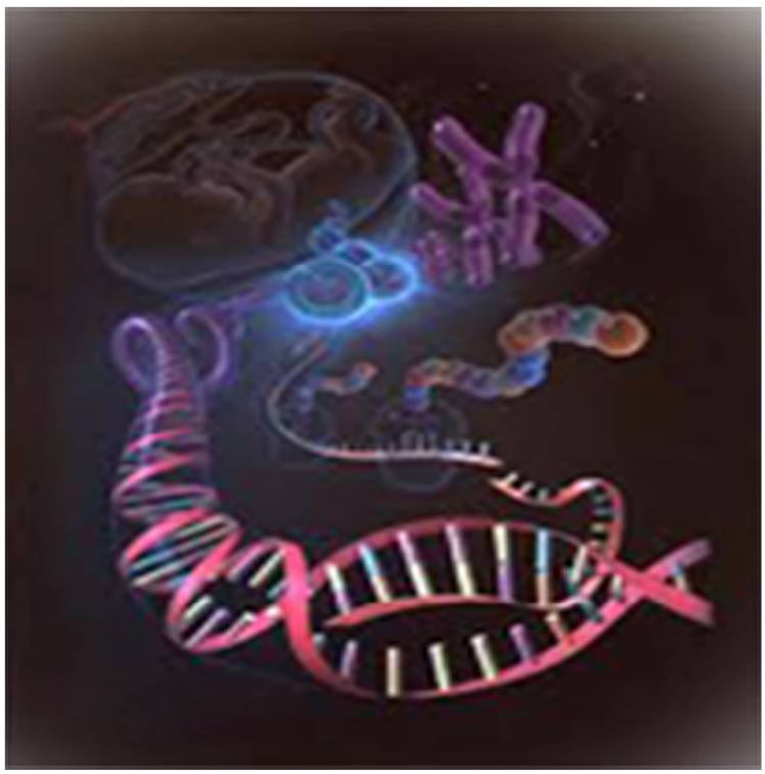
IJLBPR

ISSN 2250 - 3137
Vo1. I, No.1, January 2012

International Journal of

Life Sciences Biotechnology and Pharma Research



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

www.ijlbpr.com

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol.1, Issue. 1, January 2012

Review Article

GENETICS OF STROKE: REVIEW

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Cerebral Vascular Accident (stroke), despite remarkable progress within recent years in the field of its management and treatment, remains a major cause of mortality and morbidity in many countries. Genetics of complex diseases has known a great progress in recent years , due to technology development, but also to two major international initiatives; the Human Genome Project that has enabled the sequencing of the human genome in its entirety and the discovery of SNP (Single Nucleotide Polymorphisms), which had a great success for association studies. Genetic studies of stroke have shown that apart from rare forms with Mendelian transmission, most strokes are considered as multi-factorial disease. The genetic study of stroke is being more and more research—more than 2300 candidates for stroke polymorphisms are currently listed. These factors have generally been short-listed based on their involvement in metabolic pathways known or through studies pangenomics. The most of them have been association studies by different teams and different populations. Although most of these studies seem to focus more on myocardial infarction stroke is attracting increasing interest of researchers. The genes studied are most typically those involved in the coagulation pathway, the metabolism of homocysteine, and lipid metabolism namely FV, FII, fibrinogen, PAI1, MTHFR, ApoE and ACE. Others have been explored without consensus (Enos, PON, LPL, FGA / FGB / FGG, F7, F13A1, vWF, F12, SERPINE1, ITGB3, PLA2, ITGA2B, ITGA2, GP1BA, AGT, NOS3, LPL, PON1, PDE4D, ALOX5AP, MTR, CBS, NINJ2). The results published today are often controversial depending on the population studied, age of patients and subtypes of stroke. Some of these factors come close to the consensus than others.

Keywords: Stroke, Genetics, GWAS

INTRODUCTION

Cerebral Vascular Accident (stroke), despite remarkable progress within recent years in the field of its management and treatment, remains a major cause of mortality and morbidity in many countries.

Genetics of complex diseases has known a great progress in recent years, due to technology

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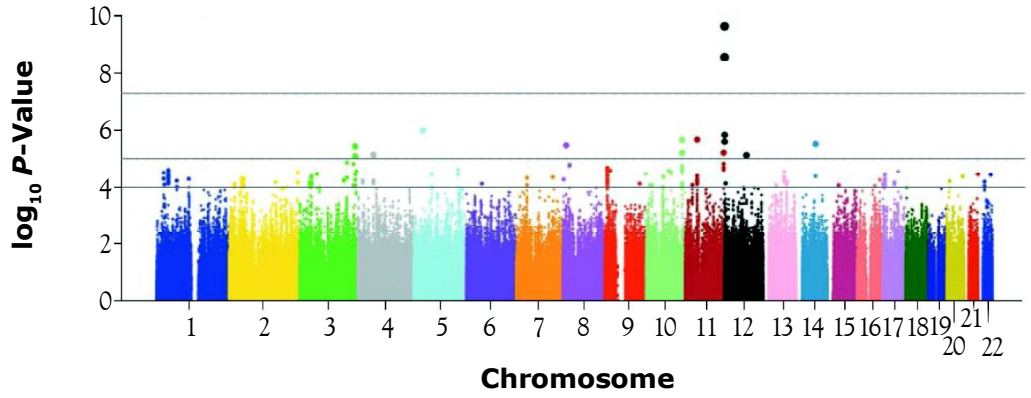
Figure 1: Results of the Association of Stroke with Snp Tested in GWAS



Note: P-Value (based on the fixed-effects model) are shown in signal-intensity (Manhattan) plots realtive to their genomic position for total stroke (Panel A) and ischemic stroke (Panel B). Within each Chromosome, the results are plotted left to right from the p-terminal end. The top horizontal line indicates the chosen threshold genomewide significance, P = 5 x 10^{-8}; the middle line indicates the threhold for P = 10^{-5} ; and the bottom line indicates the threhold for P = 10^{-4}.

GWAS Projet (2009)

development, but also to two major international initiatives; the Human Genome Project that has enabled the sequencing of the human genome in its entirety and the discovery of SNP (Single Nucleotide Polymorphisms), which had a great success for association studies (Figure 1).

The second major international initiative is the project 'HapMap', which helped to develop a haplotype map of the human genome, and allowed to describe the most common polymorphisms and the linkage disequilibrium blocks. It is currently a key resource for the search for genes associated with disease but also to response to drugs and environment.

Genetic studies of stroke have shown that apart from rare forms with Mendelian transmission, most strokes are considered as multi-factorial disease. At first, the hypothesis of a genetic origin of stroke was based mainly on observations of several family units with a non-Mendelian transmission. In the Framingham project, the existence of a family history (paternal and maternal origin) of stroke was associated with an increased risk of this disease in the descendants (Kiely K *et al*., 1993). This familial aggregation could however be only the consequence of conventional risk factors like hypertension, diabetes and hypercholesterolemia, shared between parents and their children. Currently, the strongest evidence has been reported by studying twins, or by using the animal model (Rubattu S *et al*., 1996). These studies showed that the prevalence of stroke was multiplied by five in monozygotic twins compared with dizygotic twins (Brass *et al*., 1992; Jeffs *et al*., 1997; and Flossmann *et al*., 2004).

Rare Mendelian Etiologies Responsible for Stroke

Many monogenic Mendelian diseases, autosomal dominant, autosomal recessive or X-linked may be complicated by stroke (S Jeff B, 1997), in young patients without known risk factors (Rolfs

et al., 2005; and Hess *et al*., 2006). Knowledge of these Mendelian forms is important; it helps in genetic counseling and to identify patients at a presymptomatic stage in families with risk factors, sometimes, to propose a preventive approach to treatment.

When the disease is expressed in the homozygous state, it is not uncommon that stroke occurs at an early stage (sometimes in infancy), whereas, when expressed in the heterozygous state, the consequences of the genetic defect may be mild or even indistinguishable from the effects of conventional risk factors. Homocystinuria is a good example, the genetic defect expressed in the homozygous state articulates a stroke, often in childhood. When this deficit is expressed in the heterozygous state, it contributes to the risk factor of stroke indirectly. It has been shown that a moderate elevation of homocysteine was a risk factor for stroke in adults (Perry *et al*., 1995.). Apart from this disease, sickle cell disease, Fabry disease (deficiency of lysosomal alpha-galactosidase) and mitochondrial diseases as MELAS syndrome in particular are probably the most common metabolic causes of stroke.

CADASIL: The Cerebralautosomal Dominant Arteriopathy with Leukoencephalopathy and Subcorticalinfarcts (CADASIL) is an hereditary autosomal dominant cerebral arteriolopathy, identified within the leukoencephalopathies of vascular origin since 1993. Notch 3 gene was located on chromosome 19 (Tournier-Lasserve, 1993), and the first mutations were identified in 1996 (Joutel, 1996). The disease has been described in different families of European, African, North African, American (Arcos-Burgos, 2001), Indian, Asian (Kotorii, 2001). But its prevalence remains largely under-estimated, about 1 to 24,000. Two thirds of the subjects had symptomatic transient ischemic attack. It occurs on average 42 years (Chabriat, 1995; Chabriat, 1996; and Chabriat, 1997), with extremes of 20 and 65 (Chabriat, 1995; Dichgans, 1998; and Desmond, 1999).

Sickle cell disease is a common cause of stroke in children (Switzer, 2006), either in its homozygous or heterozygous form with other hemoglobinopathies such as hemoglobin C (HbC) or *a*-thalassemia (Old, 2002). Hemoglobin S is due to a mutation by substitution of adenine for thymine in codon 6 of the b-globin gene on chromosome 11p15.4, resulting in the substitution of a valine by a glutamic acid in the protein chain. This alteration of the protein causes a deformation of the red cell that becomes sickle-shaped.

The typical atherothrombotic stroke, associated with hyperplasia of the intima, proliferation of fibroblasts and smooth muscle cells, often in the internal carotid artery and the proximal, middle and anterior portions of cerebral arteries (Stockman *et al*., 1972). Many patients develop lacunar small-vessel disease (Schatz, 2002; and Switzer, 2006), in subcortical regions. It is therefore an abnormal interaction between sickle red blood cells and vascular endothelium (Hebbel, 2004; and Switzer, 2006). The sickled red cells tend to clump together and adhere to the endothelium. The endothelial activation further promotes remodeling of the arterial wall and vascular disease.

Fabry disease is a lysosomal disease, due to a deficiency of enzyme *a*-galactosidase A (GLA). Most patients are carriers of missense and nonsense mutations, large and small rearrangements, splicing defects in the coding region of *a*-galactosidase gene on the X chromosome (GLA) (Desnick *et al*., 2001,

Schaefer , 2005; Human Gene Mutation Database). Clinical signs include a acroparesthesia, angiokeratoma, and hypohidrosis, which often develop in childhood or adolescence, before the systemic complications leading to heart failure, renal failure and ischemic stroke (Grewal *et al*., 1994; Mitsias *et al*., 1996; Crutchfield *et al*., 1998; and Rolfs *et al*., 2005). Ischemic stroke predominates in the vertebrobasilar circulation (Rolfs, 2005), but its underlying mechanisms remain unknown.

MELAS: The MELAS syndrome (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Strokelike episodes) is a mitochondriopathy caused by many mutations, transmitted by maternal inheritance in mitochondrial DNA (Pavlakis, 1984; and Martinez-Fernandez, 2001). Over 80% of patients carry the A3243G mutation, an A to G transition at position 3243 of the gene MT-TL1. MELAS syndrome is associated with various symptoms. However, in monosymptomatic case, ischemic stroke is the only existing expression (Martinez-Fernandez, 2001). The brain damage underlying the episodes of ischemic stroke in MELAS are different from those of a typical cerebral infarction, the cortex is almost always involved. In many cases, the lesions are not confined to vascular territories and there is no embolic lesions or stenoses in angiography.

Primitive Genetic Diseases of the Cerebral Vessels: an increasing number of genetic diseases of the cerebral vessels responsible for stroke has been identified within recent years. Fibromuscular dysplasia, a disease of the arteries of small and medium sizes characterized by the presence of small dilatations secondary to the destruction of the media alternating with fibrous and muscular hyperplasia of the arterial wall. The occurrence of aneurysm rupture is a relatively common event in various forms of Ehlers-Danlos disease especially type IV characterized by a deficiency of collagen type 3.

Some forms of amyloidosis are characterized by a predominantly cerebral localization of amyloid deposits in the vessels. Causing hemorrhagic or ischemic stroke, this group of disorders is genetically heterogeneous. Some are due to mutations in cystatin C, which is an inhibitor of several cysteine proteinases with a cystein group (Abrahamson, 1992). Others are due to mutations in the precursor of the beta-amyloid protein (beta-APP) (Levy *et al*., 1990)

Stroke Model of Multifactorial Disease with Polygenic Component

The genetic study of stroke is being more and more research, more than 2300 candidates for stroke polymorphisms are currently listed (Matthew *et al*., 2010). These factors have generally been short-listed based on their involvement in metabolic pathways known or through studies pangenomics. The most of them have been association studies by different teams and different populations. Although most of these studies seem to focus more on myocardial infarction stroke is attracting increasing interest of researchers. The genes studied are most typically those involved in the coagulation pathway, the metabolism of homocysteine, and lipid metabolism namely FV, FII, fibrinogen, PAI1, MTHFR, ApoE, and ACE.

Others have been explored without consensus (Enos, PON, LPL, FGA/FGB/FGG, F7, F13A1, vWF, F12, SERPINE1, ITGB3, PLA2, ITGA2B, ITGA2, GP1BA, AGT, NOS3, LPL, PON1, PDE4D, ALOX5AP, MTR, CBS, NINJ2).

The results published today are often controversial depending on the population studied, age of patients and subtypes of stroke. Some of these factors come close to the consensus than others.

Risk Factors Involved in Clotting

a. Factor V Leiden Mutation: The R506Q polymorphism in the gene of factor V or factor V Leiden, is one of the most studied variants prothrombotiquesle to the present, he is the main genetic risk factor for deep vein thrombosis, but also arterial thrombosis (Casas, 2004). Several meta-analysis were conducted to study the association FVL and stroke, the results are controversial and depends on the population studies and patient age. And associationism was absent (Paluku *et al*., 2011), few significant for Jul *et al*. (Jul, 2002; and Kim, 2003), especially when patients are older, highly significant to Casas *et al*., avecun OR of 1.33 (95% CI, 1.12 to 1.58) (Casas, 2004; and Paul Bentley *et al*.). The data published on the FV Leiden mutation showed a great diversity in geographical larépartition of this mutation, it is very common in Europeans, with a north-south gradient: 4.4% in the UK versus 1.7 % in Italy and is absent in sub-Saharan Africans and peoples of East Asia. Arab populations in the eastern basin of the Mediterranean this mutation is high, Jordan (12.3%) (AWID 1999), Syria (13.6%) (Irani-Hakim, 2000), Lebanon (14.2%) (Irani-Hakim 2000, 2002 Tamim). The prevalence decreases as the distance from these regions of the Mediterranean basin, it is present in Tunisia, Algeria, very rare or absent in Morocco (Mathonnet, Nadif *et al*., 2002). This geographical difference could be explained in partiepar the history of the region, including the presence of the Turkish XVth century (Otoman Empire) in Tunisia and Algeria, not Morocco. This change is probably related to a founder effect, which would have occurred in the eastern Mediterranean sea 21000 to 34000 years ago (Zivelin, 1997) and spread across the waves of migration to other parts of the world (Castoldi 1997; and Irani Hakim, 2000).

b. Mutation G20210A of prothrombin gene FII G20210A mutation is considered the second cause (in terms of frequency) of inherited abnormality predisposing to a risk of thromboembolic disease. This polymorphism is found in the heterozygous state in 4 to 8% of subjects with a first episode of venous thrombosis. The estimated relative risk is 2-7 times higher in carriers (De Moerloose 2000, 2001 Emmrich, Paluku *et al*., 2011). The homozygous forms are rarely observed (0.014% -0.0025%) (Poort, 1996) and risk associated with homozygosity is currently unknown. The mechanism of hypercoagulability is due to an increase in the formation of thrombine. However, the available literature on the role of the variant G20210A prothrombin gene in the pathogenesis of ischemic stroke have produced conflicting results.

c. Fibrinogen Studies have shown a strong link between high plasma fibrinogen and stroke (Wilhelmsen, 1984; and Gregory W Albers 2009). The risk was estimated 2.06 (1.83-2.33) (CO Fibrinogen Studies, 2005). This relationship is controversial in part because the levels of fibrinogen are influenced by tobacco (Wilhelmsen, 1984), obesity, diabetes, psychosocial factors, inflammation and infection. Thus, it is not clear that the plasma fibrinogen is an indicator of risk factors (Rothwell, 2004).

d. Mutations in the gene encoding the PAI1 the Plasminogen activator inhibitor type 1 (PAI1), a complex form of tPA (tissue-type plasminogen

activator), is a potent inhibitor of fibrinolysis. It was shown that a high activity of PAI1 is associated with a risk important cerebral and coronary vascular disease (PAI1) however is probably not an independent risk factor in the general population. 4G/5G polymorphism located at the PAI1 gene promoter is most frequently studied in association with the DALY but this polymorphism is often analyzed in conjunction with those of gene tPA . The data association studies Case-control were not unanimous as to the involvement of the 5G polymorphism in stroke, for a protective role against the 4G allele is demonstrated through several meta-analysis. The association between circulating levels of PAI-1 and the degree of atherosclerosis was studied, this analysis led to mixed results. The PAI-1 accumulates in atherosclerotic plaques. Indeed, the effect of a predictor of high plasma PAI-1 on the development of vascular complications is invariably attenuated after adjustment for clinical and biological markers of metabolic syndrome, whereas other risk factors for atherosclerosis affecting low circulating levels of PAI-1 (smoking, total cholesterol).

e. Molecular mechanism of C667T Variant of MTHFR gene 5,10-methylenetetra-hydrofolate reductase (MTHFR) enzyme NADPH dependent isa, which catalyzes the reduction of 5,10 - methylenetetra-hydrofolate (MTHF), the major carbon donor in nucleotide biosynthesis, to 5-MTHF, which is the predominant form of folate and the donor of the methyl radical in the reaction of the re-methylation of homocysteine to methionine. The MTHFR gene is located on a lechromosome in 1p36.3 (Goyette *et al.* In 1994). It comprises 11 exons and extends over a length of 2.2 Kb (Goyette, 1998), the gene contains no TATA box but contains several ilôts CpG very important for union sites for other transcription factors (Gaughan *et al*., 2000). C677T polymorphism, an autosomal recessive mutation, MTHFR thermolabile makes a 50% decrease in activity (Frosst *et al*., 1995). This mutation makes the protein thermolabile, and its enzymatic activity is reduced by half (Frost, 1995). The presence of the mutation in the homozygous state affects folate metabolism and induces a moderate elevation of plasma homocysteine. The C677T polymorphism of the MTHFR gene has been extensively studied in association studies with the DALYs are by far the more standardized, they are based on strict criteria such as age, subtypes of DALYs. These studies found an association with the TT genotype DALY, stronger when the subject is young, with significant or (Corin 2005, Xiao2009, KT Moe, 2008, Paluku, Nadif S. *et al* 2010). Subjects with the TT genotype have higher homocysteine levels than those with genotype CC (Brattstrom, 1998) and fewer circulating folate (McQuillan, 1999; Deloughery, 1996; Ma, 1996; and Schwartz, 1997).

f. Polymorphism insertion/deletion (I/D) ACE gene The insertion / deletion polymorphism in intron 16 of the gene for the enzyme angiotensin-converting enzyme (ACE) is one of the most common genetic variants studied in relation to atherosclerotic vascular disease. His interest is justified in ischemic stroke, since Cambien *et al*., had suggested that this polymorphism was associated with myocardial infarction (Cambien *et al*., 1992), according to a recessive model (Sharma, 1998) Association studies of this polymorphism with ischemic stroke have reported an association with a relative risk of about 1.5 to 4, other studies have failed to find significant association. This can be explained by the fact that this polymorphism is very common in the

population and up to 90% (Ueda, 1995; Catto, 1996; Pullicino, 1996; Doi, 1997; Lin, 2000; Dikmen, 2006; Gao, 2006; Pera, 2006; Tuncer, 2006).

g. The gene for apolipoprotein E (ApoE) The gene for apolipoprotein E gene is one of the most widely studied in vascular disease and neurodénératives (Eichner, 2002). Several meta-analysis conducted and reported controversial results between the ApoE4 allele and the risk of ischemic stroke, some with weak associations in patients younger than 45 years (Xi, 2009) in the Asian population (Benerje, 2007), no association in Italy (Cerrato, 2005), Turkey (Duzenli, 2004) and Taiwan (Lin HF, 2004). These results showed that the exact role of Apo E4 polymorphism in ischemic stroke is uncertain, probably due to the variability of its distribution in populations around the world. This therefore requires large samples and studies in other populations or ethnic groups.

h. The Phosphodiesterase 4D gene (PDE4D) The PDE4D is the first gene discovered by linkage Icelandic patients and described as associated with stroke, studies that followed this discovery led to conflicting results, an association was reported by (Nan Li1 *et al*. 2010) the SNPs of the PDE4D (83T / C) and IL-1 (-889C / T) associated with increased were risk for the development in Northern Han Chinese (Nan Li *et al*., 2010). It remains difficult to conclude whether or not the association and the role of the PDE4D gene in the pathogenesis of DALYs. In fact we have few studies, and they are limited to a small number of patients. It would be interesting to study larger series of different populations.Therefore, experimental studies on the function of PDE4D help to unveil the mystery. PDE4D is a large family of PDE, which are the cAMP hydrolytic enzymes and key molecules of signal transduction in several cell types, including smooth muscle cells of blood vessels. These findings also apply to ALOX5AP (5-lipoxygenaseactivatingprotein) involved in the process of vascular inflammation which gives this gene of particular interest mainly in its synergy with the processor of atherosclerosis (ElinLõhmussaar, and the Gene ALOX5AP PDE4D Gene in a Central European Population of Stroke Patients, 2005).

i. "5-lipoxygenase-activating proteingene" gene (ALOX5AP) A second positive relationship étudeIslandaise of stroke was introduced in 2004, it showed that the locus13q12 13est-linked gene with both myocardial infarction and cerebral infarction/TIA (Helgadottir A, 2004). In this chromosomal region is the gene ALOX5AP involved in the metabolism of leukotrienes, thus in atherosclerosis. The sequencing of the gene ALOX5AP revealed the presence of SNP haplotype (HAPA), associated with a risk two times higher in ischemic stroke and hemorrhagic. These results were répliquédans a Scottish study (Helgadottir, 2005) and has not been found in a study of 640 American and 97% white (Meschia, 2005).

j. Other candidate genes and pathways other candidate genes have been studied for possible association with ischemic stroke. They are listed in the web table or discussed in reviews. Among these genes, those involved in inflammation such as interleukine 1, interleukin 6, TNF, Toll-likereceptor 4, P-selectin and E-selectin, C-reactive protein in lipid metabolism such as apolipoprotein E, paraoxonase, epoxy), the release of nitric oxide. In most cases, however, the results were negative or could not be replicated in future studies.

Table 1: Final Panel of Genetic Factors With Different Degree of Association With Stroke

Type of factor	Factor	Gene	Polymorphisms	Association
Coagulation system	FactorV Leiden	F5	c.1691G>A	Possible
			c.4070A>G	Uncertain
	Prothrombin	F2	c.20210G>A	Possible
	Fibrinogen	FGA	c.4266A>G	Uncertain
		FGB	c.148C>T	Not Demonstrated
			c.455G>A	Not Demonstrated
	Factor VII	F7	A1/A2	Not Demonstrated
			c.10976G>A	Not Demonstrated
			c.323_324insCCTATATCT	Not Demonstrated
			c.402G>A	Not Demonstrated
			c.401G>T	Not Demonstrated
	Factor XIII	F13A1	c.143G>T	Not Demonstrated
	Von Willebrand factor	VWF	p.Pro564Leu	Not Demonstrated
			Sma I	Uncertain
			c.1423C>T	Not Demonstrated
			c.1793C>G	Not Demonstrated
	Factor XII	F12	c.46C>T	Uncertain
Fibrinolytic system	Plasminogen activator inhibitor 1	SERPINE1	c.675_676delinsG	Possible
			c.1053 G>T	Not demonstrated
Platelet receptor	GpIIb-IIIa complex	ITGB3	GPIIIa PLA2	Not demonstrated
			GPIIIa c.1691G>A	Not demonstrated
		ITGA2B	GPIIb p.IIe843Ser	Not demonstrated
	Gp Ia-IIa Complex	ITGA2	GPIa c.807C>T	Not demonstrated
			GPIa c.873G>A	Not demonstrated
	GpIb/IX/V Complex	GP1BA	HPA2 c.3550C>T	Possible
			VNTR	Not demonstrated
			GPIb (-5) T/C Kozak	Possible
Renin-angiotensin-aldosteron system	ACE	ACE	g.11417_11704del287	Possible
	Angiotensinogen	AGT	p.Met174 Thr	Not demonstrated
			p.Met235 Thr	Not demonstrated
Homocysteine and eNOS metabolism	eNOS	NOS3	g.3726_3834insGAAGTCTA GACCTGCTGCGGGGGTGAG	Uncertain
			c.894G>T	Uncertain
			c.786T>C	Uncertain
	Hcy	MTHFR	MTHFR c.677C>T	Possible
			MTHFR c.1298A>C	Not demonstrated
		CBS	CBS c.844_845ins68	Not demonstrated
			CBS c.833T>C	Not demonstrated
		MTR	MTR c.275A>G	Not demonstrated
Lipoprotein metabolism	APOE		$\mu 2$, $\mu 3$, $\mu 4$	Possible
		APO $\mu 2$, $\mu 3$, $\mu 4$	p.Cys112Arg	Not demonstrated
			p.Arg158Cys	Not demonstrated
	LPL	LPL	S447X	Not demonstrated
			p.Asp9Asn	Not demonstrated
			c.1127A>G	Not demonstrated
			c.93C>T	Not demonstrated
	PON1	PON1	p.Gln192Arg	Uncertain
			p.Leu55Met	Uncertain
			c.107C>T	Uncertain
Linkage-association studies	PDE4D	PDE4D	SNP 39-44-56-83-87-89	Uncertain
	5-lipoxygenase-activating-protein	ALOX5AP	HAPA	Uncertain
			SG13S106- SG13S89	Uncertain

CONCLUSION AND OUTLOOK

Strokes are very complex diseases; it is a great research model because it is the epitome of multifactorial diseases whose pathogenesis involves several risk factors constitutional, environmental, and genetic. Several epidemiological and clinical studies have been conducted, and have contributed to a better understanding of the etiology of stroke and the implementation of recommendations and preventive measures. Thus, stroke has declined relatively in industrialized countries (ref). Although the effectiveness of preventive measures remain critical due probably to the low public support campaigns for modifiable risk factors (such as smoking, alcohol, obesity, etc.), But can be done that scientists have not yet fully understood all the phenomena that explain the development of these pathologies, specially the underlying genetic. If the genetic etiology of stroke has been extensively studied in recent years many gray areas persistent. These studies were based on a classical 'candidate gene', but the scientific community was confronted with the limits of its own knowledge. Indeed, the development of stroke is determined by the complex interplay between genetic risk factors, constitutional and environmental numerous studies have elucidated in part. Only partially, especially in regard to genetic epidemiology, because the traditional approach was based on the screening of genetic polymorphisms involved in metabolic pathways known. However, the pathophysiology of stroke footprint also unknown way that only the advent of genome-wide studies 'hypothesis free' has provided a glimpse. Thus, thanks to this technological revolution since 2007, many novel polymorphisms associated with vascular disease or risk factors have been identified and new pathways identified. However, gray areas remain, including the cumulative effect of polymorphisms identified, which is currently well below the estimate of genetic heritability of risk factors, probably related to a deficiency in information clinical and genetic epistasis those relating to the phenomena, that is to say the interaction between genes. Indeed, their influence is not simply additive or dominant/recessive, and only interactive but also a systemic approach for gene networks will be evaluated. In addition, gene-environment interactions are not yet integrated into the genome-wide approaches when it is established that in some cases, the presence of fully functional gene is not sufficient for the emergence of a trait in the individual in the absence of certain environmental stimuli. Genome Wide Association Studies (GWAS) are still in their beginning and still do not have techniques complex statistics that would enable to incorporate gene-gene interactions (epistasis) and interactions in gene environment. Currently, only 12% of identified SNPs are located near protein-coding regions (Hindorff *et al.,* 2009). 40% were in intergenic regions and 40% in introns. This may mean that these areas intronic and intergenic could have a regulatory role of the expression of certain genes (Hardy *et al*. 2009). Such studies are a major challenge in terms of resources and design effort required collaboration of several centers and construction of large databases of clinical and epidemiological data.

CONFLICTS

None.

ACKNOWLEDGMENT

Many thanks to Dr. Afaf Ben Itto for reading the manuscript and for his comments to me very useful. Our work on stroke has been supported by the Hassan II Academy of Science and Technology and the Hassan II university.

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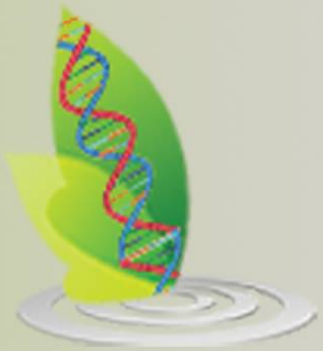

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