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Review

Genetics of ischaemic stroke in young adults

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ABSTRACT

Background: Stroke may be a clinical expression of several inherited disorders in humans. Recognition of the underlined genetic disorders causing stroke is important for a correct diagnosis, for genetic counselling and, even if rarely, for a correct therapeutic management. Moreover, the genetics of complex diseases such the stroke, in which multiple genes interact with environmental risk factors to increase risk, has been revolutionized by the Genome-Wide Association Study (GWAS) approach.
Scope of review: Here we review the single-gene causes of ischemic stroke, bringing the reader from the candidate gene method toward the exciting new horizons of genetic technology.
Major conclusions: The aetiological diagnosis of ischemic stroke in young adults is more complex than in the elderly. The identification of a genetic cause is important to provide appropriate counseling and to start a correct therapy, when available. The advent of GWAS technology, such as for other complex pathological conditions, has contributed enormously to the understanding of many of these genetic bases. For success large, well phenotyped case cohorts are required, and international collaborations are essential.
General significance: This review focuses on the main causes of genetically-based ischemic stroke in young adults, often classified as indeterminate, investigating also the recent findings of the GWAS, in order to improve diagnostic and therapeutic management.

Contents

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1. Introduction

Despite substantial progress in prevention and treatment, stroke remains a very relevant condition representing the first cause of adult disability [1], the second cause of dementia [2,3] and the third cause of mortality in developed countries [4,5]. Therefore, increasing our understanding of the risks, causes and treatment of ischaemic stroke is of great importance.

Only in Italy, every year there are about 196,000 new cases of stroke: among these about a 20% dies in the following month and about 30% survives with disabling consequences [6,1,7]. The incidence of stroke rises exponentially with age, and is quite low in young adults [8]. However, ischemic stroke is a common cause of admission of young patients in stroke units [9,10]. In particular, the yearly incidence of stroke increased from 2.4 per 100,000 for people aged 20–24 years, to 4.5 per 100,000 for people aged 30–34 years, and to 32.9 per 100,000 for people aged 45–49 years. Stroke is slightly more frequent in women aged 20–30 years and in men older than 35 years [9].

Traditional risk factors for stroke such hypertension and diabetes and pathological conditions like large extracranial and intracranial atherosclerosis, small vessel disease and atrial fibrillation, which play an important role in older patients, are much less frequent in young adults; therefore, the main clinical challenge in management of a young adult with stroke is the identification of its cause, which often (35% to 42%) remains undetermined [11].

Stroke is believed to be a complex multifactorial and polygenic disease, arising from a wide number of gene-gene and gene-environment interactions. Genetic factors could act by predisposing to conventional risk factors, by modulating the effects of those risk factors on the target organs or, conversely, by a direct independent effect on stroke risk and on infarct evolution.

The proportion of strokes of undetermined or rare causes is much higher for young adults than for elders, and in many cases underlying causes are genetic-related. The clearest evidence that genetics may cause ischemic stroke comes from monogenic forms of the disease, although these account for only a relatively small percentage of overall ischaemic strokes. In most cases, it is likely that multiple genes are involved in stroke pathogenesis acting on a wide range of candidate pathways, such as the haemostatic and inflammatory system, homocysteine metabolism, rennin angiotensin aldosterone system, and so on [revised in 12,13]. Genetic investigation of individuals who have had a stroke is a promising approach for identification of novel biological mechanisms that underlie the development of cerebrovascular disease. Thanks to modern advances in the field of stroke genetics, many cases of cryptogenic stroke have been clarified; the discovery of new pathogenetic pathways might lead in the future to the development of preventive strategies and acute treatments [14,15]. The genetic component is more prevalent in large-vessel ischemic stroke than in small-vessel or cryptogenic ischemic stroke [16], and in patients younger than 70 years of age [17]. Multicentre studies concluded that siblings usually develop the same stroke subtype. These findings have been confirmed and extended by studies in which the heritability of ischemic stroke was calculated from genome-wide data, giving estimates of 40% for large-vessel ischemic stroke, 33% for cardioembolic stroke, 16% for small-vessel ischaemic stroke, and 38% for the combined endpoint of any ischemic stroke [18,19].

In this review, the most well-characterized monogenic disorders associated with stroke will be cover. Recent advances in both common polygenic conditions associated with stroke and GWA available reports will also be presented.

2. Monogenic diseases

Monogenic diseases are responsible of about 5% of stroke cases [20]. However, the percentage is likely to be underestimated because of the diagnostic complexity and the high phenotypic variability of these conditions. There are more than 50 monogenic diseases that can cause stroke [20] [see Table 1]. Recognition of individuals and families carrying mutations causing Mendelian or mitochondrial diseases with stroke as a phenotypic manifestation remains an important challenge for clinicians. Mendelian disorders can be recognised by their familial aggregation, relatively young age of onset, more severe clinical course, and higher recurrence rates, compared with sporadic diseases. Vice versa, mitochondrial-related strokes may be maternally inherited, frequently multi-systemic and life-threatening.

Table 1
Common mutations in monogenic diseases for details see the text.

Monogenic diseases	Involved genes	Genes functions	References
MELAS	*tRNA (Leu) A3243G*	Mitochondrial tRNA	[21,22]
	tRNA (Leu) T3271C	Mitochondrial tRNA	[23]
	tRNA (Lys) A8344G	Mitochondrial tRNA	[24]
Familial hemiplegic migraine	*CACNA1A*	Encoding the alpha1A sub-unit of the voltage-gated calcium channels in neurons	[39]
CADASIL	*NOTCH3*	Unknown	[40]
CARASIL	*HTRA1*	Protease	[40]
FABRY	*α-GAL A*	Encoding α-galactosidase A enzyme	
Small vessel disease	*COL4A1*	Encoding the α1[IV]-chain of type IV collagen	[40]
HERNS	*TREX1*	Encoding three-prime repair exonuclease 1	[40]
Stroke and vasculopathy with ADA2 mutations	*CECR1*	Encoding the ADA2 protein (important for endothelial and leukocyte development and differentiation)	[63]
Homocystinuria	*Multiple genes encoding different enzymes*	Deficiencies of this enzymes can cause very high plasma concentrations of homocysteine and homocystinuria	[12]
Sickle cell disease	*Haemoglobin beta chain gene*	Encoding for beta chain of normal haemoglobin (mutation of this gene causes polymerization or aggregation of abnormal hemoglobin -. HbS - within red blood cells)	[39]
Vascular Ehlers-Danlos syndrome	*COL3A1*	Encoding collagen type III	[12]
Marfan syndrome	*FBN1*	Encoding fibrillin 1	[65]
Pseudoxanthoma elasticum	*ABCC6*	ATP-binding cassette C6	[66]

Table 2
Common variants in polygenic diseases. For details see the text.

Gene	Polymorphism	Genes functions	References
MTHFR	*C677T*	This polymorphism is associated with high levels of plasmatic homocysteine	[69]
ACE	*I/D situated in intron 16*	ACE is a membrane-bound enzyme which plays an important role within the renin-angiotensin aldosterone system	[71–75]
AGT	*M235T*	AGT is a glycoprotein substrate for renin action. This polymorphism is associated with increased blood pressure, carotid plaques and white matter lesions	[76–78]
Prothrombin gene	*c.20210G4A*	This mutation is associated with increased prothrombin levels	[79]
Fibrinogen gene	*c.455G4A*	Possible association between fibrinogen polymorphisms, high fibrinogen levels and arterial thrombosis	[13]
FV	*c.1691G4A*	This mutation leads to a p.Arg506Gln amino- acid change, which determines a resistance to aPCR (activated protein C), a stroke predisposing condition	[13]
PAI-1 (*SERPINE1*)	*4G/5G*	PAI-1 is a fast acting inhibitor of tissue plasminogen (t-PA), which plays a key role in fibrinolytic homeostasis (mutations cause increased thrombotic risk)	[80,98]
APOE	*ε2/ε3/ε4*	This polymorphisms may have an impact on total cholesterol, LDL and apoE plasma levels	[108–110]
PON1	*Q192R*	Alteration of enzyme activity associated with this SNP may influence the formation of atheromas	[114,70]
MMP-3	*5A/6A*	Homozygosity for 6A allele is responsible of a lower proteolytic activity with an increased deposition of extracellular matrix and a faster progression of the atherosclerotic plaque	[117]
EAAT2	*A-to-C change at 181 bp from the transcriptional start site*	The mutant genotype is associated with increased plasma glutamate concentrations	[137]

In Mendelian disorders, the presence of a pathogenetic mutation is usually sufficient to manifest a phenotype. However, an incomplete penetrance may be observed, and not all the subjects carrying the mutation may manifest a complete phenotype. Moreover, a clear genotype-phenotype correlation is very rarely observed in monogenic strokes. At least two genetic phenomenon can influence the phenotype in both heterozygosity and homozygosity: the already described incomplete penetrance and a variable expressivity, which refers to the type, severity and natural history of the disease. Among the factors inducing the variable expressivity, the type of allelic mutation (complete absence of the encoded protein rather than reduced function), the allelic heterogeneity (different mutations in the gene), the interaction with other genes (pleiotropy) or with environment are the most documented ones. In X-linked diseases, the clinical expression in females is possible, and the severity of the phenotype is caused by the phenomenon of X inactivation ('mosaicism').

In mitochondrial DNA (mtDNA) related diseases, the complexity of the clinical phenotypes and of the genotype-phenotype correlation is even more problematic. Cells have variable amount of mitochondria, each of which contains multiple (polyplasmy) identical (homoplasmy) copies of mtDNA. Mutations located in all the mitochondrial genomes are defined homoplasmic. Although in the recent years a new interest is growing about the pathogenic role of homoplasmic mutations (that can be responsible for heterogeneous disorders with extremely variable penetrance), they are frequently silent polymorphisms. Differently, heteroplasmic mutations, which are harboured only from a part of mtDNA genomes, are often responsible for disease phenotypes. Molecules of mutated and wild-type mtDNA coexist in the same cell and mutated genomes are distributed randomly to the daughter cells, so that their amount is different in the mitotic cycles (different mitotic segregation). Symptoms usually appear when mutated mtDNAs, following successive mitoses, will be enough to cause energetic failure (threshold effect) [12]. The percentage of mutated genomes able to give rise to the disease is different from tissue to tissue. Therefore, age of onset and clinical picture will depend on the amount of mutated genomes and their distribution in the various tissues.

2.1. Mitochondrial diseases

"MELAS" (Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes) is one of the most common maternally-inherited mitochondrial disease. A number of point mutations have been described in association with MELAS phenotype. Although some of them are located in polypeptide-encoding genes, especially ND, more frequently they arise in tRNA genes. The most frequently reported mutations associated with MELAS are the A3243G and T3271C in tRNA Leu (UUR) [21–23]. Other mtDNA mutations may cause stroke-like episodes as well, i.e. A8344G mutation [24]. MtDNA heteroplasmy should be searched in available tissues, especially skeletal muscle and urinary sediment [25]. Even if rarely, nuclear gene (i.e., POLG [mitochondrial polymerase gamma] and PEO1 [mitochondrial helicase "Twinkle"] genes) mutations can cause a MELAS-like syndrome [26,27]. Clinical features of MELAS include early onset migraine, seizures, cognitive impairment, hearing loss and stroke-like episodes; lactic acidosis is frequently associated. Aetiology of stroke-like episodes range from vasogenic oedema [28], cytopathic toxicity and hyperperfusion [29] and MRI/MR spectroscopy show lesions with no specific arterial territory distribution with a major involvement of temporal, occipital and parietal cerebral areas [30]. In MELAS patients, the most severe COX deficiency associated with the highest proportion of mutated mtDNA was observed in the walls of the leptomeningeal and cortical blood vessels, supporting the hypothesis of vascular mitochondrial dysfunction in the pathogenesis of stroke-like episodes [31]. Other features of mitochondrial disorders are muscle weakness, exercise intolerance, eyelid ptosis, short stature, pigmentary retinopathy, axonal multifocal neuropathy, sensorineural hearing loss, ophthalmoparesis, migraine, optic neuropathy, diabetes mellitus, hypertrophic cardiomyopathy and Wolff-Parkinson-White syndrome [25,32]. Typical histological findings in muscle samples are ragged red and/or COX-negative fibers. Very recently, our group, in collaboration with the Italian Network for mitochondrial diseases, observed for the first time that male gender could represent a risk factor for the development of stroke-like episodes in Italian 3243A > G carriers [32]. To date, no therapy is available to treat MELAS patients [33]. Most therapeutic strategies to treat this condition use supplements and enzyme cofactors to enhance mitochondrial metabolism and activity of the respiratory chain [34]. The treatment of choice may be represented by the combination of L-arginine (usually applied intravenously at a dosage of 0.4–0.5 g/kg in the acute phase and then switched to long-term oral supplementation) [33,35], carnitine and coenzyme Q10, plus corticosteroids for the vasogenic oedema and anti-epileptic drugs or other supportive treatments when needed [36]. Other molecules such as creatine and idebenone have been suggested to decrease lactic acid levels and reduce stroke-like episodes [37,38]. Treatment options and drugs with potential mitochondrion-toxic actions (i.e., valproic acid, metformin, linezolid, etc.) have been recently reviewed [36].

2.2. Familial hemiplegic migraine

Familial hemiplegic migraine may be responsible of stroke-like episodes particularly in childhood and in teenage years. About 50% of cases are determined by mutations in the *CACNA1A* gene, encoding the alpha1A sub-unit of the voltage-gated calcium channels in neurons. Typical clinical feature is hemiparetic, sensory, visual or dysphasic long-

lasting aura, but also basilar migraine and cerebellar ataxia is common and stroke-like episodes or coma are possible complications, even if neuroimaging (in particular MRI) does not show white matter abnormalities or sub-cortical strokes. Treatment options to fight attacks include intranasal ketamine and intravenous verapamil [39].

2.3. Cerebral autosomal dominant (CADASIL) (and recessive-CARASIL) arteriopathy with subcortical infarcts and leukoencephalopathy

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), caused by dominant mutations in the *NOTCH3* gene [40], is now recognised as the most common cause of hereditary cerebral small-vessel disease and vascular cognitive impairment in young adults [41]. The diagnosis is suspected if there is a family history and if MRI shows the characteristic confluent subcortical white matter changes extending to the temporal lobes; confirmation comes from skin biopsy and genetic testing [40,41]. Patients can clinically present with disorders ranging from migraine with aura (20-40%), ischemic events (strokes or transient ischemic attacks, 60-80%), subcortical vascular dementia, seizures and mood disturbances, despite the lack of well defined risk factors [42–44]. The estimated prevalence of CADASIL in young patients with stroke is low (0.5% of lacunar strokes; 2% in patients younger than 65 years with white matter changes) [45] but most likely is underestimated. Some modifiable vascular risk factors such as hypertension and smoking are associated with an increased probability of stroke in patients with CADASIL, suggesting that these conditions might modulate the clinical expression of the disease. Accumulation of granular osmiophilic material within the tunica media are suggestive pathological markers of CADASIL, which lead to luminal stenosis in cerebral arteries and consequent reduction in cerebral blood flow, mainly in the subcortical white matter [46]. Neuroimaging shows ischemic lesions in the basal ganglia, periventricular white matter and temporal lobes [47]. Although there is no real cure for CADASIL, prevention of ischaemic attacks is commonly based on treatment of vascular risk factors and antiplatelet drugs rather than anticoagulants because of the increased risk of cerebral haemorrhage [41]. However, the benefits of platelet antiaggregates for CADASIL have not been established yet [48].

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) or Maeda syndrome [49], due to mutations in the protease *HTRA1*, is clinically similar to CADASIL but with earlier onset (third or fourth decade of life) and systemic symptoms, including alopecia, arthropathy, and spondylosis deformans [40]. This condition is predominant in males. Brain MRI shows diffuse white matter changes and multiple lacunar infarctions in the basal ganglia and thalamus [49]. Histopatologically, small vessels in cerebral white matter and basal ganglia undergo arteriosclerotic changes resulting in luminal stenosis and loss of arterial smooth muscle, in the absence of granular osmiophilic or amyloid material [40,50].

2.4. Fabry disease

Fabry disease is a X-linked congenital lysosomal storage disorder caused by partial or total deficit of α-galactosidase A enzyme. This pathological condition presents incomplete penetrance and variable expressivity, higher in males [51], and is characterized by a progressive accumulation of glycosphingolipids (particularly globotriaosylceramid and galactosylceramide) within many tissues and cell types including vascular endothelial cells, kidney, heart and neurons, with progressive multiorgan involvement. Tissue damage is thought to be at least partly due to poor perfusion [52] and the most frequent clinical consequences are renal failure, hypertrophic cardiomyopathy, and stroke [53]. Gastrointestinal symptoms, hypohidrosis, angiokeratomas, corneal opacities (cornea verticillata), neuropathic pain are also common and may help in the diagnostic approach [52]. Neurological symptoms are wide-ranging with the most common presentation being peripheral small fiber painful neuropathy also with gastrointestinal symptoms [23,54,55]. Ischemic stroke and TIA are also common presentations, leading to cerebrovascular events at an early age [56]. The intracranial posterior arteries are frequently involved; the most prominent MRI findings are severe progressive white-matter lesions and coexistence of large-vessel and small-vessel disease with torturous and dilated large vessels (dolichoectasia) [56]. The well recognised "pulvinar sign" (T1-weighted hyperintensity in the pulvinar nucleus of thalami), when present is also suggestive of this disease [57]. Atypical neurological presentations (e.g., transient global amnesia [TGA]-like episodes) are rare but possible [58]. The diagnosis in symptomatic men can be confirmed by a deficit in serum α-galactosidase, and genetic testing, particularly in women who usually have normal α-galactosidase activity [59]. The availability of an effective therapy (α-galactosidase enzyme replacement therapy) has led to a great interest in Fabry's disease as a cause of stroke in young adults, however the risk of stroke remains substantial and management of conventional risk factors is important as well as prompt enzyme substitution therapy [60,61].

2.5. Other rare autosomal dominant small-vessel diseases

Small vessels disease associated with *COL4A1* (encoding the α1[IV]-chain of type IV collagen) mutation present with infantile hemiparesis, seizures, migraine, visual loss, dystonia, ischemic and hemorrhagic strokes, mental retardation, cognitive impairment and dementia [40]. Magnetic resonance imaging (MRI) shows diffuse leukoencephalopathy with deep white matter involvement of posterior periventricular areas, subcortical infarcts and microbleeds [62]. A subset of individuals with *COL4A1* mutations are reported to have a distinct systemic phenotype, usually with asymptomatic brain pathology, referred to as hereditary angiopathy with nephropathy, aneurysms and cramps (HANAC) syndrome. The mutations in these patients cluster in a 31-amino-acid region of the COL4A1 protein that encompasses integrin binding sites.

Hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS) is caused by mutations of the *TREX1* (three-prime repair exonuclease 1) gene, and is clinically characterised by psychiatric symptoms, dementia, subcortical strokes, and leucoencephalopathy. The onset is usually in the fourth or fifth decade of life. Ophthalmological findings – telangiectasias, microaneurysms and retinal capillary obliteration starting in the macula- are common. MRI shows contrast-enhanced lesions in the white matter of the cerebrum and cerebellum [40].

2.6. Homocystinuria

Homocystinuria represents a group of mostly autosomal recessive enzyme deficiencies (e.g. cystathionine betasynthase), which cause very high (>100 μmol/L) plasma concentrations of homocysteine and homocystinuria [12]. The disease should be considered in patients with early stroke, mental retardation, dislocation of the ocular lenses, and Marfan-like skeletal abnormalities. Homocystinuria must be distinguished from milder (15–100 μmol/L) hyperhomocysteinemia, a well documented risk factor for stroke in the general population and associated with deficient dietary B6, B12, or folate [12]. Homocystinuria can cause stroke through atherosclerosis and thromboembolism, small-vessel disease and arterial dissection. Early diagnosis of homocystinuria is essential because complications can be reduced through vitamin B6 administration [12].

2.7. Stroke and vasculopathy associated with ADA2 mutations

Loss-of-function mutations in *CECR1*, encoding the ADA2 protein, are associated with a spectrum of vascular and inflammatory phenotypes, ranging from early-onset recurrent stroke to systemic vasculopathy or vasculitis. ADA2, produced by myeloid cells, is an adenosine deaminase-related growth factor, which is important for endothelial

and leukocyte development and differentiation. Although ADA2 is not expressed in endothelial cells, in patients with *ADA2* mutations there is a defect in endothelial integrity in the small vessels as well as impairment of M2 macrophage differentiation. Deficiency of this protein may compromise endothelial integrity while polarizing macrophage and monocyte subsets toward proinflammatory cells, establishing a vicious circle of vasculopathy and inflammation. Therapeutic strategies for the treatment of patients with ADA2 deficiency require investigation. Since ADA2 is found in plasma, these patients may benefit from fresh-frozen plasma or recombinant ADA2, or, alternatively, given that monocytes and macrophages, the main producers of ADA2, are derived from bone marrow, it is possible that bone marrow transplantation or genetic manipulation of bone marrow cells might play a role in the treatment of these patients [63].

2.8. Sickle cell disease

Haemoglobin S (HbS) results from a substitution of thymine for adenine in the beta chain gene of haemoglobin. Sickle cell disease is due to a homozygous or a compound heterozygous state with HbS occurring in combination with other haemoglobinopathies [39]. The disorder is most prevalent in patients of African or African American. The mutation causes polymerization or aggregation of abnormal hemoglobin within red blood cells. Patients commonly have compensated hemolytic anemia, mild jaundice, and vaso-occlusive crises that cause excruciating pain in the back, chest, and extremities. Transient ischaemic attacks, ischaemic and haemorrhagic strokes, seizures and spinal cord vascular events occur in ≈ 25% of individuals by age 45 years [39]. Treatment options include repeated transfusions, hydroxyurea and in some instances bone marrow transplantation [39].

2.9. Disorders of the connective tissue associated with stroke

Ischemic stroke is a well-known complication of several other heritable connective tissue disorders, which can be responsible of large-vessel diseases. Here we consider only the more frequent conditions, but many other hereditary connective tissue diseases may be associated with stroke [64].

- *Vascular Ehlers-Danlos syndrome*
  Vascular type of Ehlers-Danlos syndrome (type IV) is an autosomal dominant disorder resulting from mutations in the collagen III gene (*COL3A1*), the gene for collagen type III. The more common cerebrovascular complications include intracranial aneurysms, arterial dissection, and spontaneous rupture of large and medium-sized arteries [12].
- *Marfan syndrome*
  Marfan syndrome is an autosomal dominant disorder affecting the musculoskeletal and cardiovascular systems caused by mutations in the fibrillin 1 (*FBN1*) gene. Cerebrovascular complications of the disease include transient TIAs, ischemic strokes, and subdural haematoma [65]. Associated clinical features include pectus carinatum or excavatum, reduced upper-to-lower-segment ratio or increased arm-span-to-height ratio, scoliosis, ectopia lentis, dilation or dissection of the ascending aorta, lumbosacral dural ectasia [65]. The association of ischemic neurovascular events with cardioembolism and/or aortic and cerebral artery dissection is still debated.
- *Pseudoxanthoma elasticum*
  Ischaemic stroke has also been recognised as a complication of pseudoxanthoma elasticum, a recessive disease (rarely dominant) due to *ABCC6* (ATP-binding cassette C6) gene mutations, which is associated with small-vessel disease and stenotic lesions of the distal carotid artery. Associated features include skin changes (increased elasticity and yellow-orange papular lesions), ocular abnormalities (angioid streaks) and hypertension [66].

3. Polygenic conditions

Single-gene disorders explain only a minority of stroke cases; stroke represents a complex condition, where multiple genes and the environment are involved. In this scenario, the role of a great number of candidate genes has been investigated through association studies, with controversial results [see Table 2].

In polygenic and polyfactorial diseases, multiple genetic and environmental factors are both necessary to reach a treshold level, critical for the phenotype manifestation. However, while in monogenic conditions the genetic counselling is usually sufficient to quantify the risk of inheritance of the specific disease, in a polygenic scenario this is not the case. The individual risk and the subsequent natural history of the disease cannot be predicted even knowing the single genetic variant(s), being that potentially modified by other conditions, i.e. lifestyle or comorbidities. Therefore, to date it is difficult for the clinician to establish the validity and the level of clinical applicability of the associations between such genetic factors and stroke [13].

3.1. Homocysteine metabolism

Plasma levels of homocysteine, a sulphidryl-containing amino acid derived from the metabolism of methionine, increase with some conditions such as age, vitamin B12, B6, and folate deficiency, or renal dysfunction [67]; other less clear cause of hyperhomocysteine are smoking, arterial hypertension, hypercholesterolemia, coffee and alcohol consumption [67]. As reported above, hyperhomocysteinemia is an independent risk factor for ischaemic stroke [68]. A common functional polymorphism of MTHFR, C677T, has been found to be associated with differences in homocysteine concentration (about 1.93 μmol/l between TT and CC homozygotes) [69]. Most studies show the causal relationship between homocysteine concentration and stroke. Severe hyperhomocysteinemia (4100 mmol/l), linked to homocystinuria, has been previously treated ("Monogenic Diseases" paragraph). Mild to moderate hyperhomocysteinemia (515–100 mmol/l) occurs in phenotypically normal subjects with genetic defects (heterozygosity of MTHFR or cystathionine-b-synthase –*CbS*-), acquired conditions, or an association of both. At least 60 mutations have been recognized on the *CbS* gene, of which the most common ones are the nucleotides change c.833C4T (resulting in p.Ile278Thr) and the c.919G4A (p.Gly307Ser), both in exon 8, and the splice alteration c.844 ins68. However, the most common gene variant associated with moderate hyperhomocysteinemia is the substitution c.677C4T of the gene encoding for *MTHFR*, mapped on chromosome 1p36.3, resulting in a p.Ala222Val substitution. This molecular variant is responsible for the reduction of 50% of enzymatic activity [13]. Some studies indicate a synergistic interaction between MTHFR and lipid metabolism in the development of atherothrombotic stroke [70].

3.2. Renin-angiotensin-aldosterone system

- *Angiotensin-converting enzyme (ACE)* is a membrane-bound enzyme which play an important role within the renin-angiotensin aldosterone system; therefore, it is involved in the development of hypertension, atherosclerosis and cardiovascular disease. The *ACE* gene is located on chromosome 17q23 and consists of 26 exons. An insertion (I)/deletion (D) polymorphism of 287 bp situated in intron 16 of the *ACE* gene, so-called ACE I/D polymorphism, has been described. The homozygous DD [71,72] was found associated with high ACE plasma and tissue [73]. ACE gene polymorphisms have been widely studied in stroke, after the demonstration of increased risk of myocardial infarction in DD genotype carriers [74]. However, the role of ACE polymorphism in ischemic stroke is uncertain [75].
- *Angiotensinogen (AGT)* is a glycoprotein which represents the substrate for renin action. AGT levels contribute to hypertension [76]. The gene encoding for AGT is located on chromosome 1. The most

common SNPs reported are the substitution of threonine for methionine at amino-acid position 235 (p.Met235Thr) and the amino-acid change methionine for threonine at 174. Some studies found an association between angiotensinogen p.Met235Thr and increased blood pressure, carotid plaques, and white matter lesions, suggesting that AGT gene variants could act at an intermediate phenotype level [77]. In addition, the AGT 235 T allele has been reported to contribute to salt sensitivity [78]. However, the effect of the AGT gene polymorphisms on the risk of ischemic stroke remains controversial.

3.3. Hemostasis (coagulation and fibrinolytic system)

Genes involved in the hemostatic mechanism are logical candidate genes in prothrombotic conditions of stroke [70].

- *Prothrombin*. Prothrombin (proenzyme of thrombin) is a vitamin K–dependent glycoprotein that converts fibrinogen into fibrin. The gene coding for prothrombin is located on chromosome 11p11–q12 and consists of 14 exons. Poort and colleagues [79] identified a single-nucleotide G4A transition, at position 20210 (c.20210G4A) in the prothrombin gene, associated with increased prothrombin levels. This variant has been correlated to an increased risk of venous thrombosis [80–82] and stroke [83].
- *Fibrinogen*. A glycoprotein composed of three polypeptidic chains named a, b, and g, encoded by different genes: FGA, FGB and FGG, clustered on the long arm of chromosome 4q28. This glycoprotein was considered for a long time an independent risk factor not only for stroke but also for myocardial infarction and peripheral vascular diseases [84]. The relation between the c.455G4A polymorphism and thrombotic disease is still unclear, even though a possible association between fibrinogen polymorphisms and high fibrinogen levels and arterial thrombosis has been postulated [13].
- *Factor V Leiden (FV)* is a large single-chain glycoprotein, encoded by a gene mapped on chromosome 1q23, involved in the coagulation process and regulated by activated protein C [85]. The FV gene's most studied polymorphism is the single point mutation c.1691G4A leading to a p.Arg506Gln amino acid change, which determines a resistance to aPCR (activated protein C), proved to be a stroke predisposing condition [13].
- *Factor VII (FVII)* is a vitamin K–dependent coagulation factor encoded by a gene located on chromosome 13q34, in which five polymorphisms have been identified. It is still unclear whether these polymorphisms, which are proved to be determinants of circulating FVII concentrations, are associated with arterial thrombosis or not [86,87]; to date, an association between FVII gene polymorphisms and stroke is denied [88].
- *Factors XII (FXII) and XIII (FXIII)* FXII is a plasma protein involved in intrinsic pathway of coagulation, fibrinolysis, and kinin formation. The gene for FXII, is located on chromosome 5q33-qter; a common SNP is the C-T substitution at nucleotide 46 in the 5′UTR of exon 1 [89], probably responsible for decreased FXII plasma levels. The role of this variant in venous thrombosis and coronary heart diseases is still under discussion [90–92] and further studies are needed to clarify the role of FXII gene variants in the stroke risk.

FXIII is a transglutaminase involved in the final step of coagulation cascade, which consists of two A-subunits (active site) and two B-subunits (carrier molecule), encoded by genes located on chromosome 6p25–p24 and 1q31–q32.1, respectively. Several polymorphisms of the A subunit have been described in the gene *F13A1* on chromosome 6; of these, c.143G4T seems to cause FXIII inactivation and could contribute to thrombotic disorders. However, the association between F13A1 p.Val34Leu polymorphism and ischemic stroke is still unclear [13].

- *Von Willebrand factor (*vWF) is a glycoprotein which promotes platelet adhesion and aggregation. Several variants have been identified in the *vWF* gene, which is localized on chromosome 12p13.3. However, none of these variants have a confirmed role in ischemic stroke [93].
- *Plasminogen activator inhibitor 1 (PAI-1)* is a fast acting inhibitor of tissue plasminogen (t-PA), which plays a key role in fibrinolytic homeostasis. High levels of PAI-1 have been detected in the atheromatic plaque [94] and have been linked to the development of myocardial infarction [95]. The human gene for PAI-1 (*SERPINE1*) is located on the long arm of chromosome 7. Several polymorphic loci have been described [80,96,98]; however, most of studies excluded an association between SERPINE1 variants and stroke [97,70].

3.4. Platelet glycoproteins

- *Platelet glycoprotein Ia–IIa complex (GPIa–IIa complex)* is the major platelet/collagen receptor responsible for platelet adhesion to exposed vascular subendothelium. Two SNPs in the gene for the GPIa subunit, the *ITGA2* gene, have been described: c.807C4T and c.873G4A. Another SNP, c.1648 G-A, which produces the amino-acid substitution p.Glu505Lys, is responsible for the HPA-5 platelet antigen system. This polymorphism is always present together with the c.807C4T variant, providing three different haplotypes (allele A1, 807C/Glu505; allele A2, 807 T/Glu 505; and allele A3, 807C/Lys 505). Only a few studies report an increased, although not significant, risk of stroke in young women carrying the c.807C4T polymorphism [98]; vice versa, other studies do not confirm association between *ITGA2* gene variants and stroke risk [87,99].
- *Platelet glycoprotein IIb–IIIa complex (GpIIb–IIIa complex)* is a receptor which plays a key-role in platelet activation, aggregation, and clot formation. The genes encoding for the GpIIb–IIIa complex, named ITGB3 and ITGA2B, are both located on chromosome 17. So far, *ITGB3* and *ITGA2B* genes SNPs do not seem to be associated with ischemic stroke [100,101].

c) *Platelet glycoprotein Ib/IX/V complex (GPIb/IX/V complex)* is the major platelet receptor for von Willebrand factor. Several polymorphisms have been characterized in the four genes encoding for the complex, mostly in the gene encoding for GPIba subunit (*GP1BA*), located on chromosome 17. The rs41439349 polymorphism, characterized by variable numbers of tandem repeats, cause a replication of a 13–amino acid sequence (from Serine 399 to Threonine 411) and consequently four different-sized variants D, C, B, A (in order of the increasing number of repeats, from 1 to 4 times) [102]. A second polymorphism identified in the *GP1BA* gene is c.3550C4T, resulting in a p.Thr145Met substitution linked to the human platelet antigen 2 (HPA-2) alloantigen system [103,104]. A possible association between the HPA2 SNP and stroke, in Japanese stroke patients [105,106] and in a series of 564 patients meta-analysed by Casas and colleagues [83], has been proposed. An increased risk of stroke has also been reported in −5C(T/C) carriers in both Japanese [106] and in Austrian patients [107].

3.5. Lipid metabolism

- *Apolipoprotein E (Apo E)*. Apo E modulates the metabolism of atherogenic lipoprotein particles and is involved in the process of cellular incorporation of specific lipoproteins. APOE ε2/ε 3/ε 4 polymorphism has been demonstrated to have an impact on total cholesterol, LDL and apoE plasma levels (total and LDL-cholesterol levels were the highest in apoE ε4 stroke patients and the lowest in ε2 subjects [108]). Several studies report a possible role of the ε4 allele as a prognostic genetic marker for the atherothrombotic subtype, lacunar

infarcts and carotid plaques [109,110], while ε2 allele has been reported to be associated with lower risk of carothid atherosclerosis and white matter disease [110,111,70].

- *Lipoprotein lipase (LPL).* LPL plays a key role in lipid metabolism, hydrolyzing triglycerides from chylomicrons and VLDL and removing chylomicron remnants and VLDLs from the circulation [112,113]. The *LPL* gene is located on chromosome 8p22. Few studies suggest that three LPL SNPs seem to increase the risk of ischemic stroke: a stop mutation Ser447Ter in exon 9 (S447X), the c.1127A4G in exon 6, resulting in p.Asn291Ser, and the p.Asp9Asn [13].
- *Paraoxonase (PON1).* PON family has been demonstrated to prevent lipid peroxidation and consequently exerts antiatherosclerotic effects. Alteration of enzyme activity due to polymorphisms in the PON genes may influence the formation of atheromas and thus increase stroke risk [114,70]. PON1 is a calcium-dependent serum enzyme located on HDL. The gene encoding for PON1 is mapped on chromosome 7q21.3. Two common polymorphisms in the coding region of the *PON1* gene are the p.Glu192Arg and p.Met55Leu. Even though in some recent studies PON1 seems to have a role in atherosclerosis and cardiovascular disease, it is still uncertain if those SNPs may be involved in stroke risk.

3.6. Matrix metalloproteinases (MMPs)

MMPs regulate the accumulation of extracellular matrix during tissue injury through their protolytic activity and have an important role in vascular remodeling and development of atherosclerotic plaques [115,116]. In human, increased MMP-3 and MMP-9 expression in some plaque regions has been observed [70]. The 5A/6A polymorphism in the MMP-3 promoter region is a widely studied gene locus and most studies support the hypothesis that homozygosity for 6A allele is responsible of a lower proteolytic activity with an increased deposition of extracellular matrix and a faster progression of the atherosclerotic plaque [117].

3.7. Genome-wide studies: linkage approach

Until very recently, main technique used to identify underlying genetic predisposing conditions for ischemic stroke was *the candidate gene method*: SNP(s) are identified in a proposed candidate gene, and the frequency of the SNPs in patients with stroke compared with controls was then determined [118].

The genetics of complex diseases, including stroke, has been revolutionized by the advent of the genome-wide association study (GWAS) approach [15]. This can be thought of as a large series of candidate gene studies performed in a single experiment on an array based format [119]. A great advantage of GWAS approach is that it allows associations between completely novel chromosomal loci and disease [118]. GWAS have provided the strongest evidence for loci associated with the common form of ischemic stroke. Most of the loci have been discovered in other related conditions such as atrial fibrillation, coronary disease, and coagulation, and subsequent replication in ischemic stroke patients.

Associations seem to be specific to subtypes of ischemic stroke; for example, a locus on chromosome 4q25 adjacent to the transcription factor PITX2 (codes for pituitary homeobox 2, critical for left-right asymmetry and differentiation of left atrium), found to be associated with atrial fibrillation, was subsequently found to be associated with cardioembolic stroke [120]. Rs1906591 and rs10033464 were analyzed in six studies including a total of 4199 stroke patients and 3750 controls of Caucasian ethnicity, revealing a significant relationship with cardioembolic stroke and atrial fibrillation only for the first SNP [121]; data were consistent with a following case-control study in a Han Chinese population of 1486 subjects [122].

Additionally, a locus on chromosome 16q22 involving ZFHX3 (the zinc finger homeobox protein 3) has been associated with both atrial fibrillation and cardioembolic stroke [120,123]. Similarly, a pooled analysis demonstrated that 6 SNPs in the chromosome 9p21 locus, first identified through a heart disease GWAS, are associated with atherosclerotic stroke independently of demographic variables or other vascular risk factors [124]. Genetic variants identified through GWASs of coagulation/fibrin phenotypes were subsequently examined for association with ischemic stroke. The rs505922 in the ABO gene was found to have a replicated association with large-vessel and cardioembolic stroke. More recently, GWASs of ischemic stroke have identified novel loci that were not previously identified through GWASs of other vascular diseases.

The SNP rs11984041 in HDAC9 (codes for histone deacetylase 9, chromosome 7p21) has been associated with large-vessel atherosclerotic stroke [125]. In addition, to confirm ischemic stroke loci initially identified in non-stroke GWASs (PITX2, ZFHX3 and the 9p21 locus), a meta-analysis of the METASTROKE collaboration, considering 12,389 ischemic strokes and 62,004 controls of European ancestry, confirmed the HDAC9 locus linked to atherothrombotic subtype and the PITX2, ZFH3 loci to cardioembolic subtype [126]. Moreover, HDAC9 variants seem to relate to the predisposition to atherosclerosis [118]. As with HDAC9, a novel locus associated with large-vessel ischemic stroke was first discovered in a stroke GWAS.

The rs12425791 and rs11833579 inter-genic polymorphisms on chromosome 12p13, close to the *NINJ2* gene (encoding ninjurin 2, a protein which might have a role on nerve regeneration after a damage) were associated with an increased risk for all ischemic strokes, stronger with the atherothrombotic subtype, among 19602 white subjects followed for 11 years; the replication of the association was observed testing a Dutch (for both SNPs, rs11833579 only for atherothrombotic strokes) and a North Black American (only for rs12425791) independent samples [127], although, in a meta-analysis of 18294 subjects (9358 cases, 8936 controls) from 11 studies in Asian populations, the rs11833579 didn't confirm these results, even analyzing separately Chinese from Japanese patients [128].

Still considering Chinese people, the rs2208454 (located on intron 3 of chromosome 20p12, in the MACROD2 gene, coding for O-acetyl-ADP-ribose deacetylase), which was already known to be related to MRI-detected brain infarcts [129], has demonstrated association with ischemic stroke and, in particular, with large-artery atherosclerosis, in a case-control study of 1486 subjects (712 cases, 774 controls), adjusting the analysis for age, hypertension, familial history, diabetes and gender [122].

A GWAS [130] has also been performed studying the homocysteine blood levels (after a methionine load) in 2710 people from the Framingham Heart Study (FHS) and 2100 people from the Vitamin Intervention for Stroke Prevention trial, individuating five genes associated with high homocysteine levels; thus, it was evaluated the association with incident ischemic stroke in the FHS patients, finding that only with the SNP rs2364368 in the ALDH1L1 locus (aldehyde-dehydrogenase-1 family member-1, coding for a protein which converts 10-formyltetrahydrofolate to tetrahydrofolate).

Another recent, small size case-control study in 200 Chinese Han patients has revealed two novel susceptibility loci in c-1orf156 gene (chromosome 1q24, rs10489177), with increased ischemic stroke risk in individual without hypertension and diabetes, and in XYLB gene (chromosome 3p21, rs17118), more related to individual with hypertension, non-smoker and without diabetes [131].

An overall meta-analysis of 17,970 ischemic stroke cases and 70,764 controls, recruited from WTCCC2 data and METASTROKE consortium statistics, found a novel association for rs10744777 on the chromosome 12q24, which didn't differ significantly in the single stroke subtypes [132].

Finally, GWAS have been conducted on metalloproteinases (MMP) loci: it was found a novel association between rs660599 (on MMP12

locus) and large-artery stroke in a European population of 6778 cases and 12095 controls [133]. In the first GWAS performed on pediatric stroke, 270 German families (in which both children and parents were affected by stroke) were investigated; an association between four members belonging to ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin Motifs) gene family was detected, with SNPs of ADAMTS2 and ADAMTS12 strongly associated and ADAMTS13 (this protein critical for von Willebrand factor degradation) and ADAMTS17 moderately associated with ischemic stroke [134].

Despite the above studies and the various associations with overall ischemic stroke risk and the specific stroke subtypes observed for SNPs, the meta-analysis from the CHARGE Risk Score Project [135], which included 2047 first-stroke patients from a baseline stroke-free population of 22,720, >55 years old subjects of European origin, followed for twenty years, and elaborated a Genetic Risk Score matching 324 SNPs stroke-related with 9 well-known risk factors, found only a small improvement in stroke prediction, when considering the GRS added to the Framingham Stroke Risk Score.

Thus, the NINDS Stroke Genetic Network (SiGN), created in 2009, in collaboration with the Center for Inherited Diseases Research (CIDR), recently designed a GWAS to genotype a total of 14,549 European and US stroke cases, classifying ischemic stroke subtypes with the Causative Classification of Stroke (CCS) system, reducing this way lacks of agreement between the single centres and harmonizing the results [136].

4. Genetic determinants in stroke outcome

Genetic research may be helpful not only to achieve a better understanding of susceptibility factors related to stroke, but also to determine stroke outcome, or response to specific therapies. Some polymorphisms could be associated not with an increased stroke risk but with a higher frequency of neurological deterioration in patients with acute stroke. This is the case of the polymorphism in the EAAT2 promoter, which seems to be responsible for the susceptibility to excitotoxicity after stroke. Mallolas et al. [137] found a highly prevalent polymorphism in the promoter of the glutamate transporter EAAT2 gene that abolishes a putative regulatory site for activator protein–2 (AP-2) and creates a new consensus binding site for the repressor transcription factor GC-binding factor 2 (GCF2). The mutant genotype is associated with increased plasma glutamate concentrations and with a higher frequency of early neurological worsening in human stroke.

Common genetic variation also affects the metabolism, plasma availability, or clinical response of stroke drugs. The CYP2C19*2 variant is associated with both reduced concentrations of clopidogrel in blood and increased risk of cardiovascular events in patients receiving this drug [138]. Similarly, SNPs located in A2M (rs669) and F12 (rs1801020) are associated with haemorrhagic transformation and in-hospital death, respectively, after alteplase [139]. For newer anticoagulants, variants located in ABCB1 and CES1 were shown to affect both trough and peak concentrations of dabigatran. In particular, a CES1 polymorphism significantly reduces the concentration of dabigatran in blood by 15%, and risk of bleeding was reduced of 33% [140]. However, the molecular studies related to the new anticoagulant drugs are at their beginning [14].

5. Conclusions

The aetiological diagnosis of stroke in young adults needs a different and more complex diagnostic work up than in older adults. Progress in the identification of the causes and mechanisms of stroke in young adults has been slow but constant, mainly because of technological progresses in neuroimaging and because the explosion of the molecular era. Identification of monogenic stroke is important to provide appropriate genetic counselling and, even though rarely, to start a correct therapy. Furthermore, a correct genetic diagnosis might improve clinical care, because specific preventive measures can be implemented on the basis of known natural evolution of some disorders [141].

No far from today, pharmacogenetic information to personalise therapeutic decision will be available, because common genetic variation may predict metabolism, plasma availability and clinical response of stroke drugs (eg, variant of CYP2C19*2).

Despite the many discoveries described, the main challenge for stroke genetics is to transform findings into actionable clinical methods [14].The advent of new techniques such as GWAS has contributed enormously to the understanding of the genetics of other complex disease and progress is just beginning to be made in stroke. For success, large, well phenotyped case cohorts are required, and international collaborations are essential.

Transparency document

Transparency Document associated with this article can be found, in the online version.

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