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Overview

Stroke and genetics

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INTRODUCTION

Stroke is the one of the leading cause of death and the leading cause of adult disability. Each year, about 15 million people worldwide suffer a stroke *(1)*. The current mortality rate of stoke in United States is 19.4% *(2)*. Stoke is also an important socio-economical issue.

Stroke is not a single disease but syndrome. It may manifest with different clinical picture, usually as a sudden focal deficits. Dementia, depression, epilepsy or parkinsonism may be consequence of multi-infarct lesions or the long-term stroke effects.

Not only different phenotypes but also very complex etiological mechanisms and different risk factors are stroke's constitutes.

Stroke is the consequence of sudden reduction of blood flow to the brain region. Two main different mechanisms are responsible for stoke occurrence. About 85% of stokes are ischemic but part of them results from vascular rapture – hemorrhagic strokes. But, ischemic stroke itself has many different subtype etiologies – large-artery atherosclerosis, cardioembolism, and small-vessel disease and less common artery dissection, vasculitis or coagulopathies. Etiologic stroke subtype assignment seems to be crucial for multicenter stoke studies *(3, 4, 5)*. In about 20–40% of patients stroke etiology cannot be established by routine diagnostic procedures. These strokes are classified as a cryptogenic.

Unmodified factors increasing a person's risk of stroke are: age, gender, race, ethnicity, and heredity (*6*). Modified risk factors responsible for 80–90% of strokes are well established. These are hypertension, smoking, diabetes mellitus, diet, alcohol intake, psychosocial stress, depression. Lowering high blood pressure, quitting smoking, promoting physical activity and a healthy diet could substantially reduce the burden of stroke *(7, 8)*.

Genetic factors are involved in both cryptogenic stroke and stoke of known cause. Many traditional risk factors have their own genetic predisposition. Different risk factors can lead to similar or different types of stokes. As a consequence identification of genetic risk factors is very difficult because of different etiological and pathophysiological mechanism of stroke and also most importantly gene-gene and gene--environmental interaction *(9)*. Vast majority of strokes are associated with polygenic form of the disease. Several studies indicate occurrence of unique risk loci in some types of stoke e.g. large vessels arteriosclerosis, lacunar, cardioembolic stoke and lobar or deep hemorrhage stoke (*10, 11, 12*). There are also evidences for familial risk of stroke *(13, 14)*.

Besides that, many single gene disorders are associated with stroke *(15, 16, 17, 18)*. They can be divided into vasculopathies, hypercoagulable states, connective tissue, hematologic and metabolic disorders, but

so far there are no standard classification of single gene stoke disorders. Identification of monogenic disorders responsible for stroke is very important for therapeutic approach and genetic counselling, although they are account for less than 1% of all stroke.

SINGLE GENE DISORDERS ASSOCIATED WITH STROKE

Hereditary small vessel diseases

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)

CADASIL is considered the most frequent cause of genetically determined stroke. It is a prototypical small vessel disease and a significant cause of stroke and pure vascular dementia in middle-age adults. CADASIL is caused by mutations in *NOTCH3* (OMIM:125310) gene encoding a transmembrane receptor Notch3. Notch 3 receptor is composed of a large extracellular domain linked to the intracellular domain. The extracellular domain of Notch receptor contains 34 epidermal growth factor (EGF)-like repeats. Pathogenic mutations of *NOTCH3* lead to an odd number of cysteine residues in one of those EGF-like repeats. The *NOTCH3* gene is found on chromosome 19p13.2 – p13.1. It consists of 33 exons, but only mutation in exons 2–24 encoding EGF-like repeats are responsible for CADASIL. There is strong clustering of mutation in exon 3 and 4 and about 90% of mutation can be found in exons 2–6 *(19)*. Usually CADASIL manifests with migraine headaches, mood disturbances, cognitive impairment and recurrent lacunar strokes in middle age patients, without typical vascular risks factors but with family history of stroke. MRI scans typically shows symmetrical diffuse areas of increased signal on T2--weighted images and fluid-attenuated inversion recovery (FLAIR) in periventrivular white matter, centrum semiovale, and predominantly in anterior temporal lobe and external capsule (Fig. 1, 2). T1-weihgted images usually reveal multiple lacunar infarcts and dilated perivascular Virchow-Robin spaces predominating in the basal ganglia *(20)*. Microbleeds usually located in cortical-subcortical regions, white matter, thalamus and brainstem can be seen on T2*-weighted gradient echo MR images *(21)* (Fig. 4, 5). Patognomonic finding for CADASIL are deposits of granular osmiophilic material (GOM) in the vessel wall, but their origin, chemical nature and function are still unknown *(22)*. The prevalence of the disease is unknown. More than 1000 families with CADASIL have been reported worldwide. The largest series of patients comes from France, Germany, United Kingdom, Finland, Italy and the Netherlands *(23)*. Recent study of CADASIL prevalence in an outbred Western European population in the northeast of England has shown that >1 in 13,500 individuals either have or are at risk of developing CADASIL *(24)*. There is no specific treatment for CADASIL. Identification and management of modified stroke risk factors are recommended.

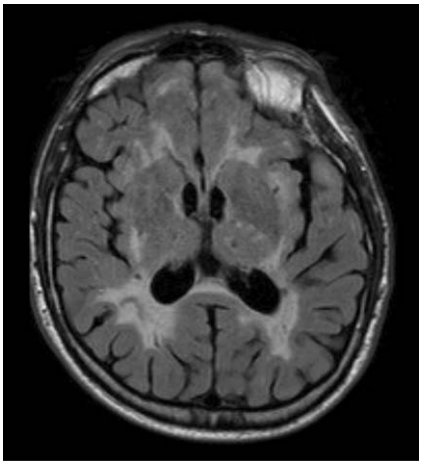

Figure 1. *FLAIR MRI of the brain showing hyperintensive confluent lesion involving white matter of the periventricular area and external capsule bilaterally in a patient with genetically confirmed CADASIL.*

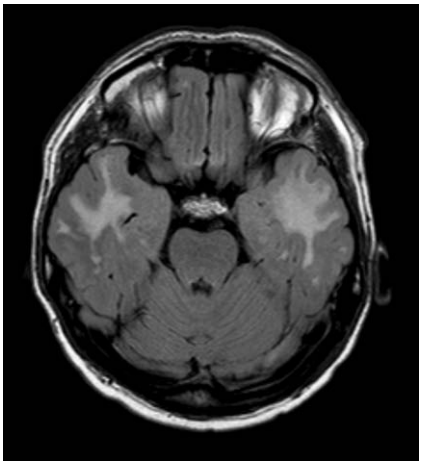

Figure 2. *FLAIR MRI of the brain showing hyperintensities involving the temporal poles in a patient with genetically confirmed CADASIL.*

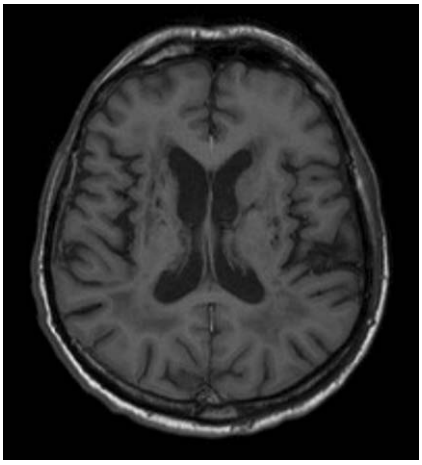

Figure 3. *MRI of the brain shows T1-hypointense lesions (lacunar lesions) in deep white matter regions in a patient with genetically confirmed CADASIL.*

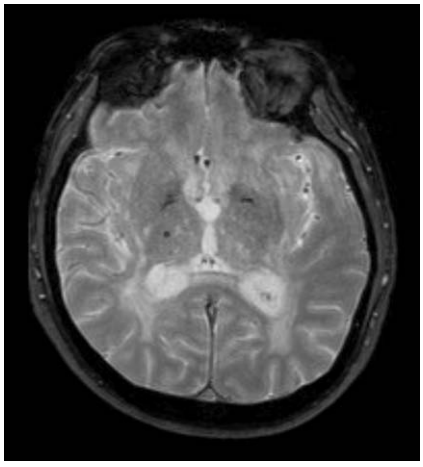

Figure 4. *T2* MRI of the brain shows microbleed in right thalamus in a patient with genetically confirmed CADASIL.*

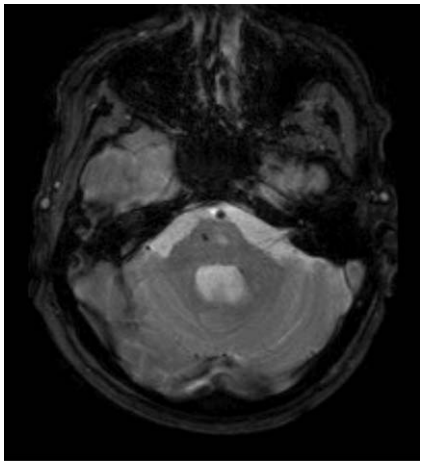

Figure 5. *T2* MRI of the brain shows microbleed in brain stem in a patient with genetically confirmed CADASIL.*

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL)

CARASIL (known as a Maeda syndrome) is a rare hereditary cerebrovascular disease caused by mutations in the *HTRA1* (OMIM:600142) gene encoding HtrA serine peptidase/protease 1 (HTRA1). This mutations induce dysregulation of TGF-ß–family signaling and as a consequence, lead to vascular injury *(25)*. Main features of the disease are similar to CADASIL features – young adult-onset stroke in the absence of common vascular risk factors, progressive motor and mental deterioration, diffuse white matter changes and multiple lacunar infarcts in the basal ganglia and thalamus on MRI imaging. In addition alopecia and spondylosis are common CARASIL manifestation. The exact incidence of the disease is unknown. Until now CARASIL has been reported in about 50 patients, most of them are from Japan. Isolated cases have been reported in China *(26)*.

Retinal Vasculopathy and Cerebral Leukodystrophy (RVCL)

Hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS), cerebroretinal vasculopathy (CRV) and hereditary vascular retinopathy (HVR) are condition which have common etiology. These three disorders have recently been considered phenotypic variants of the single autosomal dominant inherited entity – retinal vasculopathy and cerebral leukodystrophy (RVCL) *(27)*. They are caused by mutation in *TREX1* (Three prime Repair EXonulease) gene located on chromosome 3 which encode autonomus DNA 3'–5' exonulease *(28)*. TREX belongs to group of DNA nucleases witch are responsible for genome stability and are involved in DNA replication, repair and recombination *(29)*. Probably described in 2008 Hereditary Systemic Angiopathy (HSA) with cerebral calcifications, retinopathy, progressive nephropathy, and hepatopathy might be also included to RVCL spectrum *(30, 31)*.

Clinical picture of HERNS includes strokes, migraine headaches, psychiatric disturbances, cognitive impairment, visual loss and nephropathy. Signs and symptoms usually first appear in middle age. Contrast-enhancing subcortical lesions and lacunar infarcts can be seen on brain imaging studies. A pathological study shows vasculopathy with a typical multi-laminated vascular basement membrane in the brain and other organs (kidney, stomach, intestine and skin) *(32)*.

Collagen, type IV, alpha 1(COL4A1) – related disorders

COL4A1 gene encodes the alpha-1 chain of type IV collagen. COL4A1 is the major component of basement membranes of blood vessels. It forms 'chicken-wire' meshworks that provide structure and strength to basement membranes *(33)*. Mutation of *COL4A1* results in the increase of fragility of the vessel wall *(34)*. Defects of COL4A1 are responsible for familial porencephaly with white matter disease (POREN1) (OMIM:175780), small vessel disease with hemorrhages (BSVDH) (OMIM:607595) and hereditary angiopathy with nephropathy aneurysms and muscle cramps (HANAC) (OMIM:611773) *(33, 35, 36, 37, 38)*. *COL4A1* mutations may manifest with a variable phenotype including neurological features as a stroke, migraine, infantile hemiparesis, epilepsy, and systemic features including ocular, renal, and muscular involvement. But in some cases with cerebral small vessels disease no systemic features are detected *(33)*.

Fabry disease

Fabry disease is the X-linked inherited metabolic disorder caused by mutation of gene encoding α-galactosidase A (*GLA*) (OMIM:301500). This mutation induce deficiency of lysosomal alfa-galactosidase A which results in lysosomal accumulation of glycosphingolipids (particularly globotriaosylceramide) in various organs especially in vascular endothelium and smooth muscles cells leading to multi-system complications including renal, cardiac, cerebrovascular, and skin disorders *(39)*.

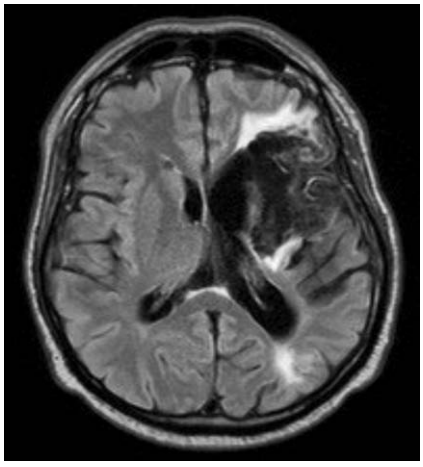

Figure 6. *FLAIR MRI of the brain demonstrates old stroke (encephalomalacia) in the left cerebral hemisphere, in the middle cerebral artery vascular territory in a patient with genetically confirmed Fabry disease.*

The symptoms of Fabry disease begin during childhood or adolescence and include skin and mucosal angiokeratomas, episodes of severe pain of feet and hands, usually triggered by fever, exercises or hot water. Ocular manifestations of the disease are conjunctival vascular abnormalities, corneal opacities (cornea verticillata), lens opacities and retinal vascular abnormalities. These changes do not usually cause significant visual impairment *(40)*. Cerebrovascular, cardiac and renal involvement usually developed later. Cerebrovascular manifestations consist of strokes in young adults, vascular dementia, MRI white matter hyperintensities due to small vessels disease, dolichoectasia of intracranial artery, especially basilar and vertebral artery. Mechanisms of brain ischemia in patients with Fabry disease are complex and enclose small and large cerebral artery disease, autonomic system, cardiac and haemostatic origin *(41)* (Fig 6).

Mitochondrial disorders

Mitochondrial disorders are an important cause of stroke especially in young patients *(42)*. They are heterogeneous group caused by dysfunction of the mitochondrial respiratory chain. Mutations in the mitochondrial DNA (mtDNA) as well as mutations in nuclear genes encoding proteins involving in the respiratory chain are responsible for the mitochondrial disorders. Mitochondrial DNA contains the information of about 37 genes: 13 genes encoding proteins involving in the oxidative phosphorylation system, 22 mitochondrial transfer RNA and 2 ribosomal RNA genes *(43)*. Mitochondrial diseases are inherited almost exclusively from the mother *(44,45)*. Oocytes and sperm have their own mitochondria and mtDNA. After fertilization zygote receives both maternal and paternal mtDNA, but during early embryogenesis sperm mitochondria disappear. This process is still poorly understood *(46, 47)*. An intriguing feature of mtDNA mutations is heteroplasmy. Each cell contains hundreds of mtDNA molecules and in one cell may coexist wild-type and mutated mtDNA. The proportions of wild-type and mutated mtDNA vary between cells and between the tissues and if it exceeds a certain threshold pathologic phenotype may appear *(48)*.

Many clinical problems are associated with the mitochondrial diseases. Organs that have high energy requirement such as brain, heart or skeletal muscles are mainly affected. Muscles involvement can manifest as weakness, cramping, muscle pain, ptosis or ophthalmoplegia. Heart blocks and cardiomyopathy are the main cardiologic complication. Diabetes, liver and renal disorders, gastro-intestinal dysfunction, visual and hearing impairments are another clinical problems. Neurological manifestation includes both central and peripheral nervous system and consists of seizures, dementia, psychiatric disturbances, stoke, stroke-like episodes, neuropathic pain and polyneuropathy *(49)*.

Stroke-like episodes are the most striking feature of MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes). It is one of the most common maternally inherited multisystem disorder with a clinical onset between the first and third decade. Only mutations in the mtDNA were reported to be responsible for the disease. About 80% of mutations are point mutation at nucleotide pair 3243 within the tRNA-Leu gene *(50)*. The nature of stroke-like episodes is still unknown. They usually occur in parieto-occipital regions or parieto-temopral regions and are not restricted to single vascular territory. They may be either symmetrical or asymmetrical and on subsequent scans may be observed mark regression of these lesions. Many molecular and cellular mechanisms leading to mitochondrial cytopathy and angiopathy have been discovered *(51)*. There is suggestion of neuronal hyperexcitability, which increases energy demand. Mitochondrial defect failure to meet these metabolic requirements especially in susceptible neuronal population and lead to cortical necrosis *(52)*. Another theory is apoptotic cell death through a progression from vasogenic to cytotoxic edema due to improper regulated hyperperfusion, which try to compensate the metabolic cell demand *(53)*. Blood-brain barrier disruption results from mitochondrial respiratory failure in endothelium of small cerebral vessels may also be involved in the mechanism underlying stroke-like episodes *(54, 55)*. It is known that mitochondrial diseases may overlap and stroke or stroke-like episodes were also reported in other mitochondrial syndromes *(56, 57)*.

Inherited connective tissue disorders

Marfan's syndrome

Marfan's syndrome is characterized by a combination of skeletal, ocular, and cardiovascular abnormalities. The frequency is estimated at 2–3/10,000. Marfan's syndrome is caused by *FBN1* gene (OMIM:154700) mutation which, results in defects in fibrillin-1. In 2004 *TGFBR2* mutation at chromosome 3 was newly identified as the cause of the Marfan's syndrome type II *(58)*. *TGFBR2* gene encodes the transmembrane receptor type II of TGFß.

TGFBR2 mutation-related disorders as familial thoracic aortic aneurysms and dissections, Shprintzen–Goldberg craniosynostosis syndrome and Loyes-Dietz syndrome share many features with Marfan's syndrome and are also associated with cerebrovascular events *(59)*. The most common neurovascular complications of Marfan's syndrome is cerebral or spinal ischemia caused by dissection of the ascending aorta, carotid and vertebral arteries or embolic strokes produced by valvular dysfunction and disturbances of cardiac rhythm *(60)*. Association of Marfan's syndrome with intracranial aneurysms is under debate *(61, 62, 63)*.

Pseudoxantoma elasticum

Pseudoxanotma elasticum (PXE) is characterized by ectopic mineralization and fragmentation of elastic fibers of connective tissues including skin, vascular walls, and the eyes. The prevalence is estimated to be about 1 in 25 0000 – 100 000 *(64)*. Females are twice as likely to be affected as males. The clinical heterogeneity is compounded by genetic complexity. Two autosomal recessive and two autosomal dominant forms have been described *(60)*. The genetic defect has been mapped to the *ABCC6* (ATP-binding cassette subfamily C number 6) gene (OMIM 603234) on chromosome 6 *(65)*. The ABCC6 protein transports substrates, which modulate arterial calcification and other phenotypic changes of PXE, but all mechanisms by which this mutations of *ABCC6* became pathogenic are still unknown. Cerebrovascular complications are consequence predominantly of large vessels disease and less frequently by small vessels disease *(66)*. Occlusive lesion of extracranial and intracranial arteries, intracranial aneurysms, cavernous fistulas and artery dissection can also be seen in patients with PXE. Multilacunar infarcts are main neurological manifestation as a consequence of hypertension associated with the disease. Typical dermatological changes are yellowich papules or plques resembling xantomas on the neck and in flexural areas. Characteristic histopathological examination is useful for diagnosis. Ophthalmological findings as peau d'orange, angioid streaks and choroidal neovascularization are typical ocular signs of PXE. Bleeding and scarring of the retina may also occur, and can lead to vision loss *(67)*. Cardiac manifestation is related to premature coronary artery disease and endocardial abnormalities. Rarely gastro-intestinal bleeding may also occur.

Ehlers-Danlos syndromes

Ehlers-Danlos syndromes (EDS) is a group of connecting tissue disorders consisting with I, II, III, and IV types. Most patients with cerebrovascular compilations have autosomal dominant type IV EDS. The disease results from mutations in *COL3A1* (OMIM:130050) (chromosome 2) encoding the pro-alpha1 chains of type III collagen that is found in extensible connective tissues such as skin, lung, uterus, intestine and the vascular system. Cerebrovascular complications may be consequence of spontaneous arterial dissection, intracranial aneurysms, subarachnoid haemorrhage and spontaneous cavernous sinus fistula *(68)*.

Some other inherited connective tissue disorders as ostesteogenesis imperfecta, neurofibromatosis (NF) and autosomal dominant polycystic kidney disease (ADPKD) can by associated with carotid dissection or intracranial aneurysms *(60)*.

COMMON POLYGENIC ISCHEMIC STROKE

Family history as a risk factor for stroke

Family history studies and twin studies indicate the role of genetic factors in the risk of stroke in general. Etiology of ischemic stroke is heterogeneous, thus genetic factors may vary by etiologic subtype. To date, most studies concern on association of the family history with the various stroke subtypes. Evidence from different researchs and meta-analyses reports a greater degree of familial aggregation and a greater inherited component of stroke among early-onset compared to late-onset ischemic stroke cases *(69, 70)*.

Family history of stroke may have a direct effect on development of stroke, but these effects may also be explained by coexistence of other phenotypes, e.g. hypertension and diabetes mellitus, that have a substantial genetic component themselves.

Recently, Framingham Study has shown the relationship between parental history of stroke and risk of stroke in offspring. Parental occurrence of stroke by age 65 years increased the risk of ischemic stroke in offspring by 3-fold. This increased risk persisted after adjustment for conventional stroke risk factors *(14)*. Another recent study revealed the impact of family history of stroke on lacunar stoke in offspring. The aim of the study was to investigated the familial aggregation of stroke in different lacunar subtypes stoke patients (patients with lacunar stroke with concomitant asymptomatic lacunar infarcts and/or diffuse white matter lesions or microbleeds). In younger probands (<65 years) there was found a higher frequency of parental family history of stroke (59% versus 20%, p=0.01) in those with asymptomatic lacunar infarct compared with probands without asymptomatic lacunar infarcts. In multivariate analysis, the strongest associations were found for parental family history of stroke (OR 6.46; 95% CI 1.96 to 21.33) *(13)*. In 2012 anther study demonstrated familial effect on ischemic stroke. Study participants who had a sibling with prior stroke were observed to have 60% higher risk of incident ischemic stroke compared with those who hadn't *(71)*.

It is known that conventional risk factors amplify the increased stroke risk associated with a positive family history *(14)*. Screening for a parental history of stroke seems to be important for stoke prevention *(14, 72)*.

Family history studies are still the most available way of measuring the inherited component of a disease and they represent the overall interaction between genetic and environmental factors. They are also important to guide future studies of genetics and epigenetics *(73)*.

Past and future of genetic studies of stroke – candidate gene study, linkage study and genome wide associated study (GWAS)

Before era of GWAS, linkage and candidate gene studies have been main investigation tools for detection the stroke genetic risk factors.

Genetic implication in common stoke is polygenic and probably there are many alleles with small effect sizes (RR <1, 5). But their impact on human health may be significant because of high population incidence of common stroke. Most previous candidate gene studies use case–control studies to investigate genetic risk factors for stroke. These studies search for differences in allele frequency between disease carriers (cases) and non-carriers (controls) with the assumption that differences infrequencies are associated with the disease outcome. Many candidate genes have been investigated and pathways involved in inflammatory, nitric oxide and endothelial function, the renin-angiotensin-aldosterone system, homocysteine metabolism, coagulation and hemostasis were included. Despite many hundreds of publications results were disappointed because only few associations have been constantly replicated *(74, 75)*. Important causes of non-replication include inadequate statistical power to detect small and moderate effects, phenotype heterogeneity, population stratification, publication bias, and multiple comparison testing *(76)*.

Genetic linkage analysis is a method that is used to associate functionality of genes to their location on chromosomes within families. This technique has been used to identify a number of genes responsible for monogenic stroke disorders, because is good at identifying genes conferring greatly increased risk. In common stroke – a polygenic complex disease, this method has been less successful. In 2003 Icelandic study have demonstrated linkage for common stroke to chromosome 5q12 and association between phosphodiesterase 4D (*PDE4D*) and ischemic stroke *(77)*. *PDE4D* gene encodes the enzyme that is the major regulator of cAMP metabolism in almost every proinflammatory and immune cells. Probably it is involved in arteriosclerosis and plaque stability and thus is suspected to be the risk gene for stroke. Numerous studies on the *PDEA4* and stroke have been carried out, with mixed results. In 2008 a meta-analysis of this studies found no evidence of association between *PDE4D* and ischemic stroke *(78)*. However this meta-analyses was restricted to single – SNP (single nucleotide polymorphism) analyses of six markers reported in a couple of studies and this seems to give no relevant data. One year later to overcome this limitations a mulit-locus Bayesian meta-analysis was performed and confirm no evidence of effect of *PDE4D* on stroke *(79)*.

The sequencing of the human genome, together with an ever expanding catalog of variations mapped as SNPs, has allowed large-scale GWAS, which have the potential to fill the gap in our understanding of the genetic basis of common diseases. GWASs provide greater power to detect small to moderate disease risk alleles than linkage studies. GWAS contrast with candidate gene studies in that no a priori biological hypothesis is needed and previously unsuspected candidate genes of interest may be identified. To carry out genome-wide association there is need of large scale cases-control studies. If certain SNPs are found to be significantly more frequent in cases compared to control, the variations are associated with the disease. However, the mechanisms of action by which associated loci influence disease or quantitative phenotypes are often unclear, because we do not know through which genes the associated variants exert their effects or because we do not know the function of these genes and do not see the clear connection to known disease *(80)*.

In 2007 the first GWSA in stroke was reported. There was found no genetic locus specifically and robustly associated with stroke *(81)*. The major limiting factor of this study was its small sample size, followed by the use of a control design with large survival and selection biases. But further study of the same group reported that chromosome 9p21.3 region represents a major risk locus for atherosclerotic stroke and the effect of this locus on stroke seems to be independent of its relationship to cardiovascular disease and other stroke risk factors *(82)*. Another GWAS identified an association in two SNPs on chromosome 12p13 with a greater risk of ischemic stroke in individuals of European and African-American population *(83)*. But one year later well-powered meta-analysis carried out by International Stroke Genetics Consortium (ISGC) and Wellcome Trust Case-Control Consortium 2 (WTCCC2) do not confirm this association *(84)*. Recently ISGC and WTCC2 published results of the largest GWAS providing investigation of stroke. In this study distinct stroke subtypes were analyzed. New association with the *HDAC9* gene and large vessel stroke was identified. Moreover, three other previously known loci were replicated on chromosome 4p23 within the *PITX* gene, 16q22 within the *ZFHX3* gene associated with cardioembolic stroke and on chromose 9p21 within CDKN2A/CDKN2B gene associated with large vessel stroke *(12)*. This publication is very valuable in stroke genetic field because it demonstrates power of very large multicenter study sample, advocates the study of distinct stroke subtype, replicates previous GWAS findings and identifies new associated genetic variant *(85)*.

CONCLUSIONS

Intensive development and improvement of genetic studies open the way to a better understanding and insight towards the mechanisms of stroke. Knowledge of the genes involved in the pathogenesis and occurrence of stroke may contribute to increase the effectiveness of prevention and stroke treatment.

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