Unravelling the Genetics of Ischaemic Stroke

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Are Genetic Factors Important in Stroke Risk?

Stroke is the third commonest cause of death and the major cause of adult neurological disability. It is a major health problem not only in the developed world, but is increasing in incidence in much of the developing world. Cerebrovascular disease is also an important cause of dementia and age-related cognitive decline, and the commonest cause of late-onset epilepsy. Conventional risk factors such as high blood pressure account for a significant proportion of stroke risk but much risk remains unexplained, and we do not understand why some individuals with risk factors such as hypertension develop stroke while others with similar risk factor profiles remain disease free. It has been suggested that genetic factors may be responsible for some of this unexplained risk, but what is the evidence for this?

Limited data from twin studies suggest stroke is more common in monozygotic compared with dizygotic twins, consistent with a role for genetic factors [1]. Considerably more data is available for family history studies, which show a family history of stroke is more common in individuals with ischaemic stroke [1]. The association is stronger for younger individuals, and those with the large artery disease and small vessel disease subtypes of stroke [2,3]. This association may represent genetic predisposition, but could also be explained by shared environmental factors. More robust data come from studying intermediate phenotypes of stroke. These are markers of disease, usually detected on imaging, which represent intermediate stages of disease pathology leading to stroke. Both twin and family studies have shown that MRI white matter hyperintensities, which usually represent small vessel disease [4], are the most heritable cerebrovascular phenotype, with a heritability (proportion of variation explained by genetic factors) estimated to be between 55% and 71% [5–7]. Carotid artery intima-media thickness, measured by ultrasound and believed to represent early stages of atherosclerosis and therefore relate to large artery stroke [8,9], has been estimated to have a heritability of 30% to 68% [10–13].

Why Should We Identify Genes for Stroke?

A small proportion of ischaemic stroke is monogenic [14]. A mutation in a specific gene results in disease, and most individuals with the abnormality are likely to develop stroke at some stage in their life. For these diseases identifying the underlying mutation allows diagnosis, prognostic information, specific treatments in some cases, and enables counselling of other family members. However, the vast majority of stroke appears to be “polygenic”; multiple genes, each likely to confer a small risk, interact with environmental risk factors to result in disease [15]. Identifying these underlying genetic risk factors may allow improved risk profiling, although as each genetic variant is likely to confer only a small risk, any useful risk prediction is likely to require a combination of multiple markers. Genetic testing for other polygenic diseases has already been developed, and indeed some gene tests for stroke are already marketed. However, their clinical relevance has been debated; most panels of genetic markers available explain only a small portion of total “genetic risk” [16]. Furthermore, their use has been questioned when clinicians already have difficulty treating known risk factors such as hypertension, which make a stronger contribution to risk, and concern has been expressed over the psychological consequences of testing. Perhaps more importantly, discovering genetic variants conferring increased stroke risk may allow new pathways involved in the pathogenesis of stroke to be identified and new treatments to be developed. This approach is just beginning to bear fruit in other “complex” diseases involving interactions between multiple genes and environment, such as macular degeneration and Crohn’s disease [17,18].

How Can We Identify Genes for Stroke?

Linkage techniques have been successfully used to identify a number of genes causing monogenic stroke. This relies on identifying associations between chromosomal markers and disease phenotype within families. Linkage is good at identifying genes conferring greatly increased risk, but has been less successful in common polygenic complex disease such as stroke. There was excitement when this approach identified the STRK1 gene in the Icelandic population as phosphodiesterase 4D [19], but this could not be replicated in other European populations [20].

Until recently the mainstay of investigating stroke genetics was the candidate gene method. Genetic variants (usually
single nucleotide polymorphisms (SNPs) are identified in a “candidate” gene that is hypothesised to be involved in stroke risk. The frequency of the SNP is compared in a group of stroke patients compared with controls. Many hundreds of such studies have been carried out in stroke with largely disappointing results [21]. This picture is common to many other complex diseases, and the reasons for lack of success have been explored in detail [21]. Important factors include small sample sizes, failure to phenotype cases accurately, and failure to replicate positive associations, combined with publication bias resulting in preferential publication of positive associations. A major problem with the candidate gene method is that associations can be identified only in genes already discovered and implicated in the disease. Completely novel genes involved in disease risk cannot be identified.

The Genome-Wide Association Study (GWAS) Approach

Recently the field of complex genetics has been revolutionised by the genome-wide association study (GWAS) approach. This approach has identified genetic loci for many other cardiovascular diseases such as coronary heart disease, diabetes, and hypertension, and is just being applied to stroke. Some novel genetic variants initially associated with other cardiovascular diseases have recently been identified as risk factors in stroke populations.
The GWAS Approach in Stroke

Stroke genetics has lagged behind that of many other complex diseases, and the GWAS approach is only just beginning to be applied. An early study in 500 individuals found no definite associations, but we now appreciate this was underpowered [25]. Larger studies are currently taking place in the UK (as part of the Wellcome Case Control Consortium 2), the US, Australia, and other countries. As yet most data, and most current studies, are from white populations. Other complex diseases have taught us that we may need very large sample sizes to identify genetic variants. For example, in hypertension, initial studies in a few thousand cases were disappointing. More recently meta-analysis of over 80,000 cases identified eight novel genetic loci [26]. The importance of robust replication prior to publication was demonstrated by the recent high-profile publication of a novel chromosome 12 locus associated with stroke identified on GWAS [27], which unfortunately could not be replicated in a sample of over 10,000 individuals, although differences in study designs—the original study was based on prospective cohorts and the replication was cross-sectional—could potentially account for some of the discrepancy [28].

Monogenic Stroke Disorders

A large number of rare monogenic disorders can cause stroke (Table 1). Some of these result in stroke as part of a systemic disorder, while others produce a clinical phenotype limited to the central nervous system [14]. Progress has been particularly exciting in cerebral small vessel disease. This causes lacunar stroke, which accounts for about 20% of ischemic stroke, and is the most common cause of vascular dementia. The most common monogenic form of small vessel disease is CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), which results from mutations in the NOTCH3 gene [33]. A striking feature of CADASIL is that, even within families, disease severity is highly variable. This variation is not accounted for by the site of the disease mutation. Confuent white matter hyperintensity called leukoaraiosis, and best seen on MRI, is a prominent feature in CADASIL. A recent study measuring leukoaraiosis volume demonstrated that the variability in leukoaraiosis volume between different CADASIL carriers has a strong genetic predisposition, with a heritability as high as 0.7 [34].

| Table 1. Monogenic or single gene disorders causing stroke, classified according to the each one’s stroke subtype. |
| Stroke Subtype | Specific Monogenic Disease |
| --- | --- |
| Small vessel disease | CADASIL |
| | CARASIL |
| | Cerebrovascular retinopathy and HERNs |
| | COL4A1 small vessel arteriopathy with haemorrhage |
| Large artery atherosclerosis and other arteriopathies | Familial hyperlipidaemias |
| | Moya-Moya disease |
| | Pseudoxanthoma elasticum |
| | Neurofibromatosis type I |
| Large artery disease—dissection | Ehlers-Danlos syndrome type IV |
| | Marfan syndrome |
| | Fibromuscular dysplasia |
| Disorders affecting both small and large arteries | Faby disease |
| | Homocysteinuria |
| | Sickle cell disease |
| Cardioembolism | Familial cardiomyopathies |
| | Familial arrhythmias |
| | Hereditary haemorrhagic telangiectasia |
| Prothrombotic disorders | |
| Mitochondrial disorders | MELAS |

HERNS, hereditary endothelialopathy with retinopathy, nephropathy, and stroke; MELAS, Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke.
as 63% [34]. This raises the intriguing possibility that other genes modulate the damage caused by the NOTCH3 mutation to modify the disease phenotype. GWAS studies are currently being planned to try to identify these modifying genes. Whether such genes modify only leukoaraiosis severity in CADASIL, or whether they could also play an important role in modifying white matter damage in response to other more common risk factors such as hypertension, remains to be determined. Similar GWAS studies are being carried out using the intermediate phenotype of white matter hyperintensity lesion volume both in normal populations and in populations with stroke and stroke risk factors. It will be interesting to compare the results of the studies in different populations.

Genes causing a number of rarer monogenic forms of small vessel disease have recently been identified. CARASIL, (cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy), which causes lacunar stroke, leukoaraiosis, and early onset vascular dementia, has been shown to result from mutations in the HtrA serine protease 1 (HTRA1) gene, which is involved in TGF-β signalling [35]. Autosomal dominant retinal vasculopathy with cerebral leukodystrophy is a microvascular endotheliothapathy presenting with visual loss, stroke, and dementia with onset in middle age. C-terminal framenshift mutations in TREX1, which encodes a DNA-specific 3' to 5' exonuclease ubiquitously expressed in mammalian cells were identified [36]. These truncated proteins retain exonuclease activity but lose normal perinuclear localization. COL4A1, a gene encoding the type IV collagen alpha 1 chain, has been found to be involved in families with autosomal-dominant porencephaly and infantile hemiparesis [37]. Patients have been reported to present with adult-onset white matter ischaemic changes consistent with small vessel disease with microbleeds in the absence of infantile hemiparesis or intracerebral haemorrhage [38]. Fabry disease is an X-linked recessive lysosomal storage disease resulting from deficient alpha-galactosidase which causes organ failure in multiple beds including the brain. Stroke may result from a number of mechanisms including cerebral small vessel disease, and confluent white matter ischaemic changes are seen on MRI. It was reported an important cause of young-onset cryptogenic ischemic stroke, accounting for 4.9% of male cases and 2.4% of female cases, although further studies are required to confirm this [39]. Therefore multiple different gene defects resulting in disruption of multiple different pathways can result in the a similar clinical phenotypes of cerebral small vessel disease.

Conclusions

In summary, genetic factors appear to be important in stroke risk, although we do not really know how important. Candidate gene studies have been largely disappointing but with GWAS technology we may well be at the dawn of a new era in stroke genetics. Future technological advances such as copy number variant determination and, whole genome sequencing are also likely to be important; the latter will allow rare variants associated with disease to be identified. Studies to date have emphasised the importance of careful phenotyping or stroke subtyping, and the experience of other complex diseases has taught us that we need large sample studies and rigorous replication of results.

Author Contributions

ICMJE criteria for authorship read and met: HM. Wrote the first draft of the paper: HM.

Five Key Papers in the Field

Matarin M, Brown WM, Scholz S, Simón-Sánchez J, Fung HC, et al. (2007) A genomewide genotyping study in patients with ischaemic stroke: Initial analysis and data release. Lancet Neurol 6: 414-420.

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