Identifying the genes involved in multifactorial stroke

H all the risk of ischaemic stroke remains unexplained by conventional risk factors and genetic predisposition has been widely speculated to account for some of this unexplained risk. Although significant progress has been made unravelling the basis of single gene stroke disorders, identifying the underlying genes for common or multifactorial stroke, for which there is no obvious Mendelian pattern of inheritance, has proved difficult. Has this situation changed with the recent publication of the first independent risk gene for common stroke?2

ARE GENETIC FACTORS IMPORTANT IN STROKE RISK?
What is the evidence that genetic risk factors are important in stroke risk? Twin studies suggest a modest genetic component, more important in younger individuals.4–5 Many studies have determined whether a family history of stroke is more common in stroke cases compared with normal controls.2 Most report an association, which is stronger both in younger individuals and with certain stroke subtypes, particularly small vessel disease (lacunar) and large vessel atherosclerotic stroke.6–9 Animal studies have implicated the existence of independent stroke genes. The spontaneously hypertensive stroke prone rat suffers both early onset stroke and larger infarcts in response to experimental middle cerebral artery occlusion. Chromosomal loci have been identified for both of these traits, although the responsible genes remain elusive.7–10 By crossing this animal with the spontaneously hypertensive rat, it has been demonstrated that these influences act independently of hypertension.

SINGLE GENE DISORDERS, STROKE, AND CADASIL
A large number of single gene disorders can cause stroke (table 1) but most of these are extremely rare. It is now apparent that CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is the most common single gene disorder leading to ischaemic stroke, and is much more frequent than was previously appreciated. True estimates of prevalence are difficult because of significant under reporting, but a minimum prevalence of 1 in 100 000 has been estimated in south east England (unpublished data). However, despite its relative frequency, recent studies have shown that CADASIL accounts for only a minority of patients with small vessel disease stroke on a population basis.11

Mutations in the notch 3 gene are associated with a systemic arteriopathy with particularly severe involvement of the cerebral small vessels.12 A combination of small lacunar infarcts affecting the white matter and deep grey matter nuclei and more diffuse chronic ischaemic changes, seen on imaging as leukoaraiosis, occur. Clinical manifestations are confined to the central nervous system; the most frequent are recurrent lacunar strokes most commonly occurring in the fourth or fifth decade, migraine usually accompanied by aura, which frequently begins in the third or fourth decade, and a subcortical dementia in the sixth and seventh decade.13–14 Depression is frequent and may precede the onset of strokes. Less common features include an acute reversible encephalopathy15 and epilepsy. All mutations reported to date result in the gain or loss of a cysteine residue, forming part of paired disulphide bonds in epidermal growth like factor repeats in the extracellular portion of the transmembrane notch 3 protein.14 This results in an epidermal growth like factor repeat with an unpaired cysteine residue, but whether this results in disease through a loss of function, or through deposition or toxicity of the notch 3 extracellular domain, is uncertain. Their stereotyped nature allows their easy identification. Genetic screening is now available in many laboratories worldwide. Mutations may occur in any one of the exons 2 to 23 encoding the extracellular portion of the protein but tend to cluster at the N-terminal end, allowing 90% to be detected by the limited screening offered by most laboratories.14–17 Skin biopsy may also be useful for diagnosis; electron microscopy shows typical granular osmiophilic inclusions adjacent to smooth muscle cells of small arteries, but estimates of its sensitivity vary from 50% to over 90%.17

The identification of magnetic resonance imaging (MRI) features, highly suggestive of CADASIL, has greatly increased recognition of the disease.18–20 Confluent involvement of the anterior temporal pole (fig 1) is rare in sporadic cerebral small vessel disease, but is present in over 90% of patients with CADASIL.18–20 Involvement of the external capsule is also common but less specific. In contrast to sporadic small vessel disease, corpus callosum involvement may occur.21 This can lead to misdiagnosis as multiple sclerosis. The combination of improved MRI diagnosis and wider availability of genetic testing has led to both increased diagnosis of CADASIL and the appreciation that the phenotype is much more diverse than originally described. Cases presenting with stroke in the eighth or even ninth decade have been reported. The phenotype may vary markedly within families, and no consistent genotype–phenotype correlations have been identified.14

IDENTIFYING GENES FOR MULTIFACTORIAL STROKE
Identifying the genetic predisposition to multifactorial stroke has proved a harder task. It is usually assumed that such influences are polygenic although the number of responsible genes is unknown. Such genes could act by predisposing to conventional risk factors, by modulating the effect of conventional risk factors, or as independent risk factors for stroke. Almost all studies of polygenic stroke to date have employed a candidate gene methodology. The frequencies of polymorphisms (DNA sequence variants) in already identified genes are compared between stroke cases and controls. A large number of candidate genes have been studied, but until recently no robust replicable associations had been reported. This is common in the genetics of many other complex diseases. It is possible that genetic influences are so small that they cannot be detected, but many previous studies have had serious methodological limitations. Sample sizes have been small with most well below those recently estimated as necessary to detect even moderate genetic risk factors for stroke.22–24 Multiple hypothesis testing and publication bias are both likely to have contributed to spurious associations. These biases can

Acknowledgments

Abbreviations: CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; IMT, intima media thickness; MRI, magnetic imaging resonance; PDE, phosphodiesterase
be limited by replication of positive associations in a second independent population, but this has been rarely performed in stroke studies.

An important consideration, ignored in many studies, is the heterogeneity of stroke. Stroke describes a syndrome of different pathophysiological processes all resulting in the common end point of focal cerebral ischaemia. Eighty-five per cent have ischaemic aetiology and 15% haemorrhagic. Even among patients with ischaemic stroke, a number of different pathophysiological processes are responsible, including cardioembolism, large vessel atherosclerosis with thromboembolism, and small vessel disease. Pathophysiological mechanisms and underlying genetic influences may differ for the different subtypes. Two recent family history studies have found associations with large vessel and small vessel disease stroke, but not with cardioembolic stroke.22 23 Associations were much stronger for younger stroke cases. Sample size calculations revealed that, if a gene predisposed to one particular subtype, studying younger patients with that subtype compared with unselected ischaemic stroke would allow associations to be identified with a tenth or less of the sample size.8 If progress is to be made it is vital that future studies take these considerations into account. Identifying large numbers of younger patients with specific subtypes is likely to require collaborative national, and even multinational, efforts.

**THE USE OF INTERMEDIATE PHENOTYPES**

The complexity of stroke has led to the increasing use of intermediate phenotypes. These represent end points part way along the process towards completed stroke. Fewer genes are likely to be involved, perhaps increasing the chance of their successful identification, and the use of intermediate phenotypes overcomes the problem of covert cerebrovascular disease whereby a “control patient” may be due to suffer stroke in the near future. They offer greatly increased statistical power because of the use of a continuous variable and can be measured in a community population, both at baseline and following a time interval, to determine associations with disease progression; however, it is important to remember that implicated genes should then be tested for association with the end point of stroke. Both carotid intima media thickness (IMT) and plaque, as an intermediate phenotype for large vessel disease, and white matter hyperintensities on MRI as a marker for small vessel disease, have been widely used. Carotid IMT can be imaged non-invasively using ultrasound and estimates correlate well with histological measurements of IMT.24 Elevated carotid IMT has been shown to be an independent predictor of both stroke and myocardial infarction risk25 26 and has a high heritability.26 27 Recent studies have shown associations with a number of candidate genes,28 including associations with genes encoding proteins involved in the inflammatory response. Strong interactions were found with conventional “pro-inflammatory” risk factors, including smoking29 and alcohol.30 These studies

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**Table 1**  **Monogenic causes of ischaemic stroke**

<table>
<thead>
<tr>
<th>Disorders</th>
<th>Gene/chromosomal location responsible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small vessel disease</td>
<td>notch 3 gene</td>
</tr>
<tr>
<td>CADASIL</td>
<td>Unknown</td>
</tr>
<tr>
<td>Cerebroretinal vasculopathy and HERNs</td>
<td>3p21.1-21.3</td>
</tr>
<tr>
<td>Large artery disease</td>
<td>Various</td>
</tr>
<tr>
<td>Moyamoya disease</td>
<td>3p24.2-26 and 17q25</td>
</tr>
<tr>
<td>Pseudoxanthoma elasticum</td>
<td>ABCC6 gene</td>
</tr>
<tr>
<td>Neurofibromatosis type I</td>
<td>NFI gene</td>
</tr>
<tr>
<td>Disorders affecting both small and large arteries</td>
<td></td>
</tr>
<tr>
<td>Fabry disease</td>
<td>α-galactosidase A gene</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>cystathione β synthase gene</td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>MTHFR</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Haemoglobin S and SC</td>
</tr>
<tr>
<td>Cardiomyopathies: primary/secondary familial dysmyopathies</td>
<td>Various</td>
</tr>
<tr>
<td>Protein C, S</td>
<td>Protein S and C genes</td>
</tr>
<tr>
<td>Antithrombin III deficiency</td>
<td>Antithrombin III gene</td>
</tr>
<tr>
<td>Familial antiphospholipid syndrome</td>
<td>Unknown</td>
</tr>
<tr>
<td>Activated protein C resistance</td>
<td>Factor V Leiden mutation</td>
</tr>
<tr>
<td>Arterial dissection</td>
<td>collagen type III gene</td>
</tr>
<tr>
<td>Ehlers-Danlos syndrome type IV</td>
<td>Unknown</td>
</tr>
<tr>
<td>Fibromuscular dysplasia</td>
<td>fibrillin-1 gene</td>
</tr>
<tr>
<td>Marfan syndrome</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial disorders</td>
<td>Mitochondrial DNA mutations</td>
</tr>
</tbody>
</table>

Disorders are listed according to postulated disease mechanisms and examples of each disease mechanism are given. Single gene disorders causing conventional risk factors for stroke, such as hypertension, will also result in stroke but are not included. Details of specific diseases are available in reviews.3 32

CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CARASIL, cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy; HERNs, hereditary endotheliopathy with retinopathy, nephropathy, and stroke; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke like episodes; MTHFR, methylene tetrahydrofolate reductase.
emphasise the importance of taking gene-environment interactions into account when designing genetic studies. Whole matter hypertensin on MRI have been studied in a number of population studies, particularly the Austrian Stroke Prevention Study. Recent neuropathological data suggest that confluent asymptomatic lesions represent small vessel disease and progress rapidly, whereas twin studies show a high degree of heritability. A series of studies have implicated the angiotensinogen gene acting independently of hypertension. PHOSPHODIESTERASE 4D: A NEW GENE FOR MULTIFACTORIAL STROKE A major limitation of candidate gene studies is that completely novel pathophysiological processes cannot be discovered. Linkage based approaches have been applied to many complex diseases such as hypertension and ischaemic heart disease but have, on the whole, been disappointing. A similar approach applied to Icelandic stroke patients, taking advantage of the country’s extensive genealogical database, identified a locus for a gene for common stroke on chromosome 5q12. Initial analyses suggested that it was a risk factor for ischaemic stroke but not for myocardial infarction. Further analyses just published suggest the responsible gene is phosphodiesterase 4D (PDE4D). Although no causative mutations have yet been identified, an at risk haplotype spanning the 5’ end and the promoter region was associated with an approximately three times relative risk of the combined end point of carotid and cardioembolic stroke, compared with a protective haplotype. No association was found with small vessel disease stroke. Associations were independent of conventional risk factors. The action of phosphodiesterase 4D is poorly understood, but this class of enzymes is involved in the selective degradation of second messenger cAMP, which has a central role in signal transduction and regulation of physiological responses. In vascular smooth muscle cells, low cAMP levels lead to cell proliferation and migration that is mediated in part by PDE4D. In addition PDE4D belongs to a large superfamily of phosphodiesterases, which have already been successfully targeted to treat diseases like asthma and inflammation. The association between PDE4D and stroke now needs replication in independent populations. The Icelandic population is a relative genetic isolate, and it remains to be determined whether the association is equally relevant in other populations. Furthermore, the lack of initial linkage with myocardial infarction, and the subsequent identification of a gene associated with carotid disease and cardioembolism, which might be expected to share similar pathogenetic mechanisms to myocardial ischaemia, is surprising. However, if confirmed, this may represent a completely new pathophysiological process causing stroke and could open the way for novel therapies.

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REFERENCES


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