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Genetics of cerebrovascular disease - current understanding and future direction

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A stroke is defined as 'rapidly developing clinical signs of focal or global disturbance lasting 24 hours or longer or leading to death with no apparent cause other than of vascular origin'. Yet stroke should not be considered a single disease, but rather a syndrome of disparate processes all leading to cerebral damage as a consequence of disruption to cerebral blood flow. While these processes may share some obvious risk factors, more and more evidence is emerging that distinct stroke subtypes have distinct conventional and genetic risk factor profiles. While conventional risk factors profiles can be identified and managed clinically, often prior to a cerebrovascular event, genetic risks are only now beginning to be investigated and understood.

The evidence for a genetic basis to stroke comes from twin studies [1, 2], family history studies [3, 4] and animal models of stroke [5, 6]. Combined, these studies suggest that as much as 50% of stroke risk may be attributable to genetics, although identification of the genes responsible for this risk in common sporadic stroke has proved difficult. The one area where there has been success in identifying the genetic basis of stroke is the single gene disorders, most noticeably cerebral autosomal dominant arteriopathy with subcortical arteriopathy and leukoencephalopathy (CADASIL) [7]. Rather than being due to highly penetrant single genes, however, common sporadic stroke is thought to be due to multiple genes each exerting a small increase in risk, with an individuals risk profile being different combinations of genetic and environmental risks. This presents novel challenges in disease risk identification, not only because of the small increase in risk attributable to any individual genetic variant, but also because these genetic variants may display incomplete penetrance and population stratification, which act to further mask the impact a particular variant may display. This problem is not unique to stroke research, however, and recent successes in other diseases with a polygenic basis provide both hope and direction to researchers investigating the genetics of cerebrovascular events.

Single gene disorders in stroke

CADASIL remains, at the time of writing, the only form of isolated stroke to display familial patterns of inheritance in which the responsible gene has been identified [7]. Several other conditions in which stroke is a secondary clinical presentation have known genetic causes (Table 1) but these account for a very small minority of stroke cases presenting to frontline medical services.

Table 1

Rare single gene disorders with stroke as a primary or secondary clinical characteristic in which the causative gene has been identified.

Disorder	Gene	Mechanism of action	Type of stroke
CADASIL	Notch3	Pure stroke syndrome affecting small cerebral vessels	Small vessel
Ehlers-Danlos syndrome type IV	Collagen 3A1	Collagen disorder, 10% of patients show neurovascular complications	Large vessel disease
Marfan syndrome	Fibrillin	Musculoskeletal disorder, 4% show neurovascular complications	Large vessel disease
Pseudoxanthoma elasticum	ABCC6	Connective tissue disorder with high prevalence of cardiovascular complications	Large vessel disease
Fabry disease	α galactosidase A	Lysosomal enzyme deficiency leading to damaged vascular endothelial cells	Large and small vessel disease
Sickle cell disease	Haemoglobin S	Stroke, TIA or neurological complications present in up to 25% of cases	Large and small vessel disease
Hereditary hemorrhagic telangiectasia	Endoglin and ALK1	Vascular dysplasia with variable expressivity leading to venous malformations	Embolitic stroke

The frequency of CADASIL is typically between 0.5% and 2% of all strokes depending on the age of the cohort investigated, being more frequent in strokes under the age of 65 years [8]. Commonly presenting in the 40s, MRI scans show characteristic changes with a combination of lacunar infarcts and white matter high signal or leukoaraiosis often involving the anterior temporal pole and external capsule. Due to mutation in highly conserved cysteine residues in the Notch3 gene that prevent homo-dimerisation [8] clinical diagnosis of CADASIL is now confirmed on the basis of gene screening. Yet despite over 50 different mutations having been reported, no clear genotype-phenotype correlations have been identified. Given the rarity of CADASIL, and this lack of genotype-phenotype correlation, the utility of knowing the genetic basis of the condition largely lies in confirmation of diagnosis after an event and in screening immediate family members. The relevance to the remaining 99% of stroke of polygenic origin is minimal.

Genetics of common stroke

A search of the literature reveals no shortage of efforts to identify the genetic basis of stroke, with, until recently, the humble candidate gene study and the family based linkage study leading the way. Despite these efforts, however, little progress has been made for sporadic polygenic stroke. Stroke genetics has lagged behind the genetics of more 'glamorous' diseases such as heart disease and breast cancer due mainly to small study sizes, poor phenotyping and mixed populations. Although hampered in part by the late age of onset of stroke, and in part to a lack of unified direction, stroke genetics took a major boost in 2003 with the identification of PDE4D (phosphodiesterase 4D) as the first gene identified as a risk factor for sporadic stroke [9]. A regulator of cyclic AMP, PDE4D was proposed to control the level of smooth muscle cell proliferation and immune function in vessels, thereby leading to increased or decreased atherosclerosis and hence ischaemic stroke risk. Attempts to replicate these findings were mixed, however, with many researchers rushing to confirm the findings in their own populations. A meta-analysis in 2008 suggested that PDE4D was likely to have little to no impact on stroke risk, although a population specific effect in the Icelandic population remains a faint possibility [10].

Other candidates for sporadic stroke have included members of the leukotriene biosynthesis pathway, genes involved in controlling the response to inflammation, genes for lipid handling and clearance, blood clotting, blood thinning and vessel remodelling, among others. Despite intensive investigation no single gene has yet been conclusively shown to contribute unequivocally to sporadic stroke risk via a candidate gene or familial linkage based study. There may be light at the end of the tunnel however. Taking a lead from other common polygenic diseases, stroke genetics research has gone 'high-tech' and embraced the emerging technology of genome wide association (GWA) to examine huge numbers of genetic variants in thousands of stroke cases in a single experiment.

Current stroke genetics research

A candidate gene study, in which a genetic variant or single nucleotide polymorphism (SNP) is chosen on the basis of prior evidence and then examined in cases and controls, is a hypothesis driven experiment. Variants are selected on the basis of prior evidence, or suspected involvement of a gene in a disease process. The technique of genome wide association (GWA) is essentially the same technique, but at much higher density. Rather than looking at a single variant, GWA looks at multiple variants at the same time – up to 1 million at a time – in a non-hypothesis driven experiment. We are assuming a disease has a genetic basis, but make no prior assumption as to where that likely genetic variant is in the genome. By looking at enough points in the genome and combining the results with some powerful statistics, the theory is that we will, if we look at enough variants, find one close to the genetic change causing disease. Made possible only through recent advances in understanding the human genome sequence and its inherent variation through such efforts as the HAPMAP project (www.hapmap.org), the technique of GWA has revolutionised the search for low penetrance alleles in common diseases.

One of the first uses of GWA in disease gene identification was for complement factor H (CFH) in age-related macular degeneration [11]. Examining 116,204 genetic variants in 96 cases and 50 controls revealed a single variant in the CFH gene at a significance of $p=1 \times 10^{-7}$ which increased the risk of disease seven fold. Interestingly this gene is in a region previously identified from family based linkage studies. GWA really found favour however in a large Wellcome Trust funded study examining seven common diseases including coronary heart disease and diabetes in 2000 cases of each disease and 3000 shared controls. This single study examined 500,000 genetic variants in the 14,000 cases, identifying 24 independent associations with the seven diseases [12]. A further 54 loci were identified with suggestive significance ($p=10^{-5}$ – $p=10^{-7}$) that are likely to be additional disease susceptibility loci. This single study proved both proof of concept and opened the floodgates for GWA as a mainstream technique provided large numbers of cases could be collected and analysed.

A full GWA in stroke has yet to be conducted, although several efforts are currently in progress (see below). What has emerged so far in stroke research has been a replication of findings from related diseases such as coronary heart disease (CHD) and atherosclerosis. Three reports detailing GWA in CHD all identified a significant locus on chromosome 9p21 [13-15]. This locus has now been replicated in a number of related diseases, including stroke [16, 17] with highly significant findings, although no specific mutations have been identified. Two genes in the region remain plausible candidates however, CDKN2A and CDKN2B, which are both tumour suppressors and regulators of cellular proliferation. Intensive efforts are continuing to identify disease susceptibility loci in this region.

GWA in stroke has lagged behind other diseases, in part due to the late onset of the condition making extensive collection of samples difficult, and in part due to the heterogeneity of the disease requiring exhaustive and expensive investigation to confirm diagnosis. Two small scale efforts - the Framingham 100k scan project which included some stroke cases, and preliminary findings from the examination of 400,000 variants in 250 ischaemic stroke cases [18], have reported, although full results are not currently available.

The most significant advance in stroke genetics has been the establishment of a worldwide network of stroke genetics researchers to form the International Stroke Genetics Consortium (ISGC) (www.strokegenetics.org). This comprises researchers from the United States, Europe, Africa and Australia combining cases to further investigate the genetic basis of ischaemic and haemorrhagic stroke. Currently under way is a GWA on over 4000 ischaemic stroke cases, with replication funded in a further 10,000 cases. With early results expected in spring 2009 stroke is at least catching up with the better recognised diseases in terms of both funding and research.

Concluding remarks

From a medical care point of view, the genetic basis of common sporadic stroke can, for the moment, be largely ignored in terms of both diagnosis and treatment. That genetics has an impact on stroke aetiology is not in doubt, as the rare single gene disorders demonstrate. Where a greater understanding of the genetic basis of the condition is likely to be felt first, however, is in risk factor

management. Screening a patient for genetic variants which alter lipid profiles, stickiness of platelets or ability of blood vessels to remodel in response to atherosclerosis are all as likely to be more beneficial than genetic influences on the actual cerebrovascular event. Similarly recovery after stroke, the likelihood of secondary events and the response to medication are further instances where understanding the genetics of stroke are likely to show a noticeable patient benefit. Though each genetic risk identified is likely to be small, multiplication of these risks within an individual who carries multiple risk alleles allows a significant individual increase in risk to become manifest. New technologies such as GWA are now being applied to identify these variants, and only with their discovery can therapeutic strategies be implemented to combat them.

Although the adage of 'prevention is better than cure' still holds true, stroke and cardiovascular disease remains the biggest single killer in the western world. Hopefully with an understanding of the genetic basis of the condition we will be able to implement new and improved therapeutic interventions targeted at the molecular level, rather than treating the consequences of disease as is all too common at the current time.

Key Learning Points

- Up to 50% of ischaemic stroke risk is attributable to genetics.
- Rare Mendelian stroke due to single genes accounts for less than 2% of all ischaemic strokes.
- Genetic risk factors are unlikely to be identified using traditional candidate gene approaches.
- New technologies such as GWA are currently being used to identify genetic risks for common sporadic cases of ischaemic stroke.

References

1. Brass LM, Isaacsohn JL, Merikangas KR, Robinette CD. A study of twins and stroke. *Stroke* 1992; 23: 221-3.
2. Bak S, Gaist D, Sindrup SH, Skytthe A, Christensen K. Genetic liability in stroke: a long term follow up study of Danish twins. *Stroke* 2002; 33: 769-74.
3. Jousilahti P, Rastenyte D, Tuomilehto J, Sarti C, Vartiainen E. Parental history of cardiovascular disease and risk of stroke. A prospective follow-up of 14371 middle-aged men and women in Finland. *Stroke* 1997; 28: 1361-6.
4. Liao D, Myers R, Hunt S, et al Family history of stroke and stroke risk. The Family Heart Study. *Stroke* 1997; 28: 1908-12.
5. Jeffs B, Clark JS, Anderson NH, et al. Sensitivity to cerebral ischaemic insult in a rat model of stroke is determined by a single genetic locus. *Nat Gen* 1997; 16: 364-7.
6. Rubattu S, Volpe M, Kreutz R, Ganten U, Lindpaintner K. Chromosomal mapping of quantitative trait loci contributing to stroke in a rat model of complex human disease. *Nat Gen* 1996; 13: 429-34.
7. Kalaria RN, Low WC, Oakley AE, et al. CADASIL and genetics of cerebral ischaemia. *J Neural Transm Suppl* 2002; 63: 75-90.
8. Joutel A, Corpechot C, Ducros A, et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature* 1996; 383: 707-10.
9. Gretarsdottir S, Thorleifsson G, Reynisdottir ST, et al. The gene encoding phosphodiesterase 4D confers risk of ischaemic stroke. *Nat Gen* 2003; 353: 131-8.
10. Bevan S, Dichgans M, Gschwendtner A, et al. Variation in the PDE4D gene and ischaemic stroke: a systematic review and meta-analysis on 5200 cases and 6600 controls. *Stroke* 2008; 39: 1966-71.
11. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science* 2008; 308: 385-9.
12. Wellcome Trust Case Control Consortium. Genome wide association study of 14,000 cases of seven common diseases and 3000 shared controls. *Nature* 2007; 447: 661-78.
13. McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. *Science* 2007; 316: 1488-91.
14. Helgadottir A, Thorleifsson G, Manolescu A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science* 2007; 316: 1491-3.
15. Samani NJ, Erdmann J, Hall, et al. Genomewide association analysis of coronary artery disease. *N Engl J Med*. 2007; 357: 443-53.
16. Matarin M, Brown WM, Singleton A, Hardy JA, Meschia JF, ISGS investigators. Whole genome analyses suggest ischaemic stroke and heart disease share an association with polymorphisms on chromosome 9p21. *Stroke* 2008; 39: 1586-9.
17. Gschwendtner A, Bevan S, Cole J, et al. Sequence variants on chromosome 9p21 confer risk of atherosclerotic stroke. *Annals of Neurology* 2009 (in press).
18. Matarin M, Brown WM, Scholz S, et al. A genome-wide genotyping study in patients with ischaemic stroke: initial analysis and data release. *Lancet Neurology* 2007; 6: 414-20.