How Stroke Genetics is impacting on clinical care: now and the future

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Abstract

The last decade has seen a transformation in our understanding of the genetics of stroke. However, a commonly asked question is how do these advances impact on the practising clinician now, and what are their implications for clinical care in the future? This review outlines the major recent advances in stroke genetics, and addresses four questions most relevant to the clinician: What do we now know about the genetic architecture of stroke? When and how should genetic testing be used to identify monogenic stroke? How can genetic information be applied to risk stratification and prevention? And what therapeutic implications are emerging from genetic discoveries? It highlights the success of genome wide association studies in stroke, and how these have transformed how we understand the genetic architecture of stroke. An increasing number of monogenic forms of cerebral small vessel disease are being identified and guidance of when and how to test for them is covered. Polygenic risk cores are gaining increasing attention. How these can be applied and the challenges in their use are discovered. Lastly the impact of genomics on stroke therapeutics, whether by identifying new targets, assessing causality using techniques such as mendelian randomisation, or pharmacogenomics are discussed.

Non-standard Abbreviations and Acronyms

GWAS	genome wide association studies
cSVD	cerebral small vessel disease
WMH	white matter hyperintensities
CADASIL	Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy
CARASIL	Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy

Introduction

The last decade has seen a transformation in our understanding of the genetics of stroke. We now know that apparently sporadic stroke has a significant heritable component, perhaps between 25 to 50%.(1,2) Large genome wide association studies (GWAS) have identified 89 or more genetic associations for sporadic stroke(3,4), and over 100 for MRI markers of cerebrovascular disease.(5,6,7) Increasing numbers of single gene disorders causing familial stroke have been discovered, and unexpectedly we now appreciate that mutations in these genes are much more frequent than we had thought. However, a commonly asked question is how do these advances impact on the practising clinician now, and what are their implications for clinical care in the future?

This review outlines the major recent advances in stroke genetics, organised around four questions most relevant to the clinician: What do we now know about the genetic architecture of stroke? When and how should genetic testing be used to identify monogenic stroke? How can genetic information be applied to risk stratification and prevention? And what therapeutic implications are emerging from genetic discoveries? (Figure 1)

What do we now know about the genetic architecture of stroke?

GWAS have been the main engine of discovery for the common variant architecture of stroke. Successive waves of increasingly large collaborative efforts have dramatically expanded our knowledge.(3,4,8,9) The 2022 GIGASTROKE cross ancestry meta-analysis, encompassing over 1.6 million participants including 110,182 stroke cases, identified 89 independent risk loci, 61 of which were novel, associated with stroke and its subtypes.(3) Importantly, the effect sizes were largely consistent across ancestries, supporting the generalisability of findings, though the need for more diverse population studies remains pressing.

While some variants predisposed to all stroke subtypes, often through shared risk factors, a striking finding was that the genetic architecture of different ischaemic stroke subtypes was distinct. For example, a variant in *HDAC9* was strongly associated with large artery, but not with cardioembolic stroke or small vessel, stroke,(9) while other variants are exclusively associated with either cardioembolic stroke or small artery stroke.(4,8) These findings emphasise that distinct molecular mechanisms underlie different types of ischemic stroke, and therefore subtype

specific therapies may be necessary. The genetic information can also be used to ask specific questions about pathophysiology. For example, gene variants associated with thrombosis and a thrombotic gene risk score, are associated with both large artery and cardioembolic stroke, but showed no association with small artery stroke.(10,11) This is consistent with our prior knowledge that thromboembolism is important in both large artery and cardioembolic stroke, but suggests it is less important in small artery stroke. This provides potential therapeutic insights into the pathogenesis and treatment approaches for small artery stroke, although these of course need to be confirmed in randomised clinical trials.

For most of the twentieth century stroke was treated as a largely non-genetic disease, although some twin and family histories late in the 1990s and early 2000s suggested a significant genetic component.(12,13) GWAS studies allow estimates of heritability and significant heritability has been demonstrated for all major stroke subtypes, ranging between 20 and 40%. (1,2)

When and how should genetic testing be used to identify monogenic stroke?

For the practising clinician, the most immediately actionable advance is the clearer guidance on which patients warrant genetic evaluation. Most cases of stroke are multifactorial, related to a variety of cardiovascular risk factors in combination with polygenic predisposition. However, a small number are caused by monogenic disorders, in which a mutation in a single gene results in disease. Most of these cause a specific stroke subtype, and the most common monogenic subtype of stroke presenting to stroke physicians and neurologists is small vessel-related stroke.(14) Monogenic cerebral small vessel disease (cSVD) can cause not only lacunar stroke, but also intracerebral haemorrhage (ICH), and more chronic ischemic changes seen on MRI including white matter hyperintensities (WMH) and cerebral microbleeds.(15)

By far the most common monogenic form of cSVD-related stroke is Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), an autosomal dominant disease caused by *NOTCH3* mutations. *NOTCH3* encodes a transmembrane protein, and all mutations are highly stereotyped resulting in a gain or loss of cysteine in one of the EGFR (epidermal growth factor-like repeats) in the extracellular portion of the protein.(16) The first feature is often migraine, usually with aura (in 90%), which is present in about two thirds of patients.(17) Most patients with classical CADASIL will develop lacunar stroke at some time in their life, usually after 40 years, but increasing numbers of cases are seen in which stroke occurs later, for example, in the 70s. A recent study following prognosis over 23 years found the median stroke-free survival time was 53 years in those recruited before 2016, and 60 years in those recruited since 2016.(18) Cognitive impairment becomes increasingly common with age, with some degree of impairment being present in about half of individuals in their 50s to 60s. The same study reported median dementia-free survival time was 77 years in those recruited since 2016, and 68 years in those recruited before 2016.(18) Other features include encephalopathy, apathy, and depression. (19)

The second most common monogenic from of cSVD is due to autosomal dominant mutations in the *HTRA1* (HtrA Serine Peptidase 1) gene, and is increasingly referred

to as CADASIL2. An autosomal recessive disease due to *HTRA1* mutations, Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), has been recognised for some time.(20) It is very rare and most cases have been reported in Japan and the Far East. In addition to early onset stroke and dementia, extracerebral manifestations are also common including alopecia and lumbar disease. (20). In 2015 it was reported that a single *HTRA1* mutation can also cause cSVD, that is in an autosomal dominant pattern. (21) It is now recognized as the most common cause of monogenic cSVD after CADASIL, and is widespread among many different populations.(22) Clinical features are similar to CADASIL including migraine with aura, encephalopathy, younger onset lacunar stroke, and early onset vascular dementia.

Anther rarer cause of monogenic CSVD is in *COL4A1/A2*, an autosomal condition characterised by not only ischaemic stroke and WMH but also ICH and cerebral microbleeds.(23) Other very rare forms of familial cSVD, which are unlikely to be seen by most stroke physicians, include CARASAL (cathepsin A-related arteriopathy with strokes and leukoencephalopathy) due a dominant variant in the *CTSA* gene (24), and cSVD associated with autosomal dominant *LAMB1* mutations.(25)

Deciding which patients to test for monogenic causes of stroke can be challenging, particularly as the age distribution for monogenic lacunar stroke and sporadic lacunar stroke overlap considerably. Useful clinical pointers include early onset stroke, migraine with aura, and importantly a family history of stroke or dementia. When taking family history, careful note should be made of other neurological conditions such Alzheimer's dementia and multiple sclerosis, which may in fact have represented vascular dementia and ischemic white matter changes respectively.

Brain imaging, particularly MRI, Is especially useful in diagnosis of CADASIL. WMH show a predilection for the anterior temporal pole and WMH in this location have 90% specificity and sensitivity for the disease,(26) although WMH also occur widely throughout the white matter. (figure 2) Confluent changes in the external capsule are also frequent, although less specific in differentiating CADASIL from sporadic SVD.(26) Unlike sporadic cSVD, involvement of the corpus callosum is common in CADASIL. This is also seen in demyelination, probably one reason why some cases of CADASIL are misdiagnosed as multiple sclerosis. MRI scans in CADASIL2 can look similar to that in CADASIL with anterior temporal pole involvement being frequent, and the two can only be reliably differentiated on genetic testing. Once a diagnosis is suspected on clinical history and brain imaging, confirmation is by genetic testing which is now most commonly performed using next generation sequencing gene arrays which can screen for other monogenic forms of stroke at the same time as CADASIL.

Whilst there is no specific treatment for CADASIL yet available, cardiovascular risk factors particularly smoking, hypertension and diabetes, have been associated with more rapid disease progression and epidemiological data suggests control of these risk factors is beneficial.(19, 27) For this reason, careful risk factor control is recommended not only for patients who have suffered stroke, but also for family members who carry the gene but have not yet suffered symptoms. A new diagnosis of a monogenic form of cSVD should also be followed by referral to genetics to enable family counselling. This is particularly important with the increasing

availability of prenatal genetic diagnosis, which allows individuals carrying the gene to use IVF based techniques to ensure that their child is unaffected. Useful guidance on diagnosis of monogenic cSVD and its management are provided in European Academy of Neurology consensus recommendations.(15)

It is hoped that the future will see specific treatments for diseases such as CADASIL. Genetic approaches such as antisense oligonucleotides (28) and small interference RNA (siRNA) (29), enable reduced gene expression and protein levels, and might reduce the NOTCH3 aggregation seen in CADASIL. These techniques have been successfully used in other neurological and systemic genetic diseases. Immunisation against the NOTCH3 deposits is being explored, currently in animal models, to improve clearance of the aggregates.(30)

Recent data has questioned how we think of the distinction between monogenic and multifactorial stroke. Epidemiological studies reported the prevalence of CADASIL as about 4 in 100 000 in the United Kingdom. Europe. (31) However, recent studies examining the frequency of typical cysteine changing *NOTCH3* mutations in publicly available databases reported a much higher frequency of approximately 2 per 1000 in Europe, and up to 10 per 1000 in parts of the Far East.(32) These early studies were performed on anonymous sequencing datasets, and the clinical correlates of these apparently asymptomatic mutations could not be determined. However, studies in databases such as UK Biobank, which have accompanying clinical and imaging information, have not only confirmed these findings but also revealed that these mutations are associated with an increased risk of stroke, as well as vascular dementia, and a higher prevalence of MRI-markers of cSVD.(33,34) Why some patients with N*OTCH3* mutations develop severe symptomatic disease, and others develop milder disease or no symptoms at all remains uncertain. Mutation location, with mutations affecting the proximal epidermal growth factor repeats (EGRF) domains 1-6 being associated with milder phenotypes(35), is an important factor as are cardiovascular risk factors, but both fail to account for much of the variance. Other modulating genes have been suggested to play a role.(36) This is an area of considerable research interest. A similar situation has been reported for *HTRA1* mutations. Autosomal dominant *HTRA1* is rarer than CADASIL but typical mutations have been reported in about 1 in 1000 individuals in UK Biobank.(34) Therefore, it appears as if monogenic cSVD mutations are much more common than previously appreciated, and may play a role in the burden of apparently sporadic cSVD.

The unexpected frequency of these mutations in the general population can also cause problems for the practising clinician. Particularly for the more distal *NOTCH3* mutations associated with lower disease severity, their detection does not always mean that they will present with symptoms, making providing advice risk prediction for individual patients challenging. The lower risk associated with these mutations can also present challenges for family testing. For example, in someone with one of these lower risk mutations is it reasonable to progress to prenatal genetic diagnosis? We need better ways of predicting risk in individuals taking into account not only mutation site, but also other potential risk indicators. Models are being addressed to address this (37), but more work in large international datasets is required to provide better prediction in individuals.

How can genetic information be applied to risk stratification and prevention?

For the majority of stroke patients, stroke arises not from a single gene defect but from the cumulative effect of hundreds of common variants, each conferring a tiny increment of risk. These can be aggregated into a polygenic risk score (PRS), which has begun to attract serious clinical interest. In GIGASTROKE, a polygenic score predicted ischaemic stroke.(3) However, when one looks at the results in more detail the increased risk prediction conferred by a higher PRS was modest. For example, in GIGASTROKE the degree of prediction of a PRS based on GWAS significant risk loci from the cross-ancestry all stroke meta-analyses, to identify individuals who are at higher risk of all ischaemic stroke after accounting for established risk factors was determined. Compared with patients in the lowest PRS tertile, patients in the top PRS tertile had an adjusted HR of 1.35 (95% CI = 1.16–1.58) ,whereas those in the middle tertile had an adjusted HR of 1.13 (95% CI = 0.96–1.33, P = 1.4×10^{-4}.(3) This indicates that being in the upper tertile only increased risk by 35%, much less for example than a diagnosis of hypertension. A major reason underlying this limited additional risk provided by such stroke PRSs is that the total variance in stroke risk explained by the GWAS results is currently low, and of the order of only a few precent. If this proportion could be increased the level of prediction a PRS conferred would increase proportionally. Including rare variants, for example the *NOTCH3* and *HTRA1* variants discussed above, can increase the level of prediction,(34) but it needs to be increased much further for clinical application. A further limitation is that most studies have found the additional prediction provided by a PRS over that provided by conventional risk factors alone is small.(38)

Even for diseases in which the PRS is based on GWAS data which explain more of the variance in disease risk, their use is controversial. As highlighted in recent European Guidelines, “enthusiasm for PRS use has grown rapidly but has arguably outpaced rigorous evaluation of clinical utility. Indeed, PRS are commercially available at present and being used by some clinicians and patients without clear guidance when they may be helpful or in whom.”(39) Furthermore many of PRSs have been developed in populations of European ancestry, and need developing and validating in other ethnicities. (40)

An additional key question is whether conveying the results of a PRS to a high risk patient will alter their behaviour. We all know how difficult it is to get some high risk patients to alter their behaviour, and that the control of established risk factors in patients at risk of stroke is often suboptimal. In coronary artery disease (CAD) a small randomized study reported that disclosure of a CAD PRS increased the participants’ perception of personal control, information seeking, and sharing, and led to statins being more often initiated as the result of the shared decision-making process between the physician and the participant. (41) In contrast, in an open randomized trial of blood donors, disclosure of CAD risk information, either based on clinical or polygenic risk factors, followed by web-based lifestyle advice did not affect physical activity or other factors impacting on CAD risk, e.g. serum lipids, weight, or diet. (42). It will be critical to learn from future studies whether and when the knowledge of a PRS alters behaviour, and in what settings. Given the current lack of evidence and somewhat contradictory results, larger and more definitive studies

including randomized trials are needed to quantify the potential benefits of PRS in relevant clinical scenarios.(39) Such studies are taking place in cardiovascular disease more widely. For example, the CVRISK-IT trial is a multicentre, open-label, randomised controlled study designed to enhance cardiovascular disease prevention through precision-based risk assessment and targeted communication strategies. A total of 30,000 adults aged 40-80 years without previous vascular disease or diabetes are being recruited across Italy.(43)

What therapeutic implications are emerging from genetic discoveries?

Perhaps the most transformative impact of stroke genetics on clinical practice, although largely indirect for the practising clinician, will be in drug discovery. Genetics can help in a number of ways. Firstly, a novel genetic association can lead to a completely new therapeutic target resulting in a novel treatment for stroke. Secondly techniques such as Mendelian randomization (MR) can be used to assess whether a particular pathway is causally related to stroke and therefore provide support that treating it will have a therapeutic effect.

In terms of new drug targets, an often cited example relevant to cardiovascular disease, is the development of PCSK9 inhibitors as a novel treatment to lower cholesterol.(44) Rare gain-of-function mutations were associated with both raised LDL-cholesterol as well as premature CAD. Conversely common loss-of-function variants, which reduce PCSK9 activity, were associated with lower LDL-C and significantly reduced CAD risk, This led to the development of PCSK9 inhibitors which were demonstrated in randomised clinical trials to result in both reduced LDL-C, and reduction in future cardiovascular events. These inhibitors are now in widespread clinical use, primarily in patients who are statin-intolerant or cannot reach their LDL-C goals with traditional treatments.(44)

Another example at an early stage in development is the histone deacetylase 9 (*HDAC9*) gene. An early stroke GWAS identified a variant in the *HDAC9* gene as a risk factor for large artery stroke (9), and subsequently coronary artery disease, but not for non-atherosclerotic stroke subtypes. HDAC9 expression is elevated in human atherosclerotic plaque, while in animal and cellular models, reducing HDAC9 protein is associated with reduced disease.(45) The association raises the possibility that inhibiting HDAC9 may provide a novel treatment approach for atherosclerotic cardiovascular disease. This is supported by intervention studies demonstrating HDAC9 inhibition reduces atherosclerosis in animal and cellular models. Indirect data supports such an approach in man.(45) The antiseizure drug sodium valproate, which has nonspecific HDAC inhibitory properties, both inhibits atherosclerosis in animal models and is epidemiologically associated with reduced stroke and myocardial infarction risk in man. (45) Both repurposing of sodium valproate, or development of novel more specific HDAC9 inhibitors could represent a novel treatment to prevent atherosclerotic stroke.

Novel treatment derived from genetic insights may be particularly important for cSVD. Despite casing a quarter of all ischaemic stroke (lacunar stroke), many ICHs, and being the most common pathology underlying vascular cognitive decline and dementia,(46) there are no proven treatments beyond risk factor modification.(47) A major reason for this has been a lack of understanding of the underlying

pathophysiology. GWAS and other 'omic studies are providing completely novel insights into what causes cSVD, and identifying potential therapeutic targets. A converging theme is the importance of disruption of the extracellular matrix and associated proteins, together referred to as the matrisome.(48) This a key component of the neurovascular unit, a functional complex in the brain composed of neurons, glial cells, endothelial cells, pericytes, and the extracellular matrix. It maintains central nervous system homeostasis by regulating cerebral blood flow and controlling the blood-brain barrier integrity. Disruption of this unit can result in both impaired cerebrovascular reactivity/autoregulation, as well as blood brain barrier permeability which are both believed to play in cSVD pathogenesis. A number of potential therapeutic targets are being followed up based on insights from both monogenic and polygenic cSVD.(49) For example HTRA1 appear to paly a key role in cSVD risk, and better understanding of the underlying molecular mechanism are pointing to potential novel treatment approaches.(50)

MR is being increasingly applied to gain further insights into pathophysiology and treatment approaches. This technique uses genetic variants as instrumental variables to approximate the effect of an exposure to seek evidence for a causal association with stroke. Numerous MR studies have been published in stroke and have confirmed the importance of conventional cardiovascular risk factors such as blood pressure.(51) They are increasing being used to screen potential treatments, and to gain insight into whether targetable risk factors are casually related. It has been estimated that genetic support of drug effects could double the success rate of clinical trials (52), and pharmaceutical companies are increasingly integrating genomics-based strategies into drug discovery pipelines. MR techniques can also be applied using genetic variants as instrumental variables which approximate the effect of exposure to a particular drug.(53)

Pharmacogenomics: Genotype-Guided Treatment

A more immediate application of genetics to individual patient care is in pharmacogenomics. The best-established example in stroke is CYP2C19 genotyping to predict clopidogrel response. Clopidogrel requires hepatic activation by CYP2C19 to exert antiplatelet effects.(54) Approximately twenty percent of individuals of East Asian ancestry, and a smaller proportion of European ancestry carry loss-of-function variants in CYP2C19 that substantially reduce active drug levels. In a secondary analysis of the CHANCE trial, loss-of-function allele carriers of the CYP2C19 polymorphism did not benefit from dual versus single antiplatelet therapy after minor stroke or transient ischemic attack for the prevention of stroke recurrence.(55) This was confirmed in CHANCE-2 in which 6412 minor stroke and TIA patients with CYP2C19 loss-of-function alleles were randomized between clopidogrel or ticagrelor for 90 days; all also received aspirin for 21 days. Stroke occurred within 90 days in 6.0% in the ticagrelor group and 7.6% in the clopidogrel group (hazard ratio, 0.77 [95% CI, 0.64–0.94]; P=0.008).(56) Guidelines, including the usually conservative English National Institute of Clinical Excellence (NICE) have recommended CYP2C19 polymorphism testing in patients with a past history of stroke and TIA, with the replacement of clopidogrel with an alternative agent such as ticagrelor if the polymorphism is detected, and suggested it is cost effective.(57,58) Point-of-care testing kits for CYP2C19 genotype testing are now available. However, to date there has been variable take up of CYP2C19 genotyping in stroke practice.

Another example, although perhaps less relevant due to the widespread adoption of direct oral anticoagulants recently, is warfarin dosing. CYP2C9 and VKORC1 variants influence warfarin metabolism and sensitivity respectively, and pharmacogenetic dosing algorithms incorporating these genotypes plus CYP4F2 can improve time-in-therapeutic-range. However, randomised trial evidence has not consistently shown superiority of genotype-guided warfarin dosing over standard clinical algorithm (59) and it has not been widely adopted in clinical practice.

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Figure 1

Examples of how genetic advances may impact on clinical care

Figure 2

Imaging appearances of CADASIL. T2 weighted MRI scan showing anterior temporal pole white matter hyperintensities (left), external capsule and periventricular WMH (centre) and a susceptibility weighted sequence (SWI) showing microbleeds in the thalamus bilaterally.

