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REVIEW

Monogenic causes of stroke: now and the future

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Abstract Most stroke is multifactorial with multiple polygenic risk factors each conferring small increases in risk interacting with environmental risk factors, but it can also arise from mutations in a single gene. This review covers single-gene disorders which lead to stroke as a major phenotype, with a focus on those which cause cerebral small vessel disease (SVD), an area where there has been significant recent progress with findings that may inform us about the pathogenesis of SVD more broadly. We also discuss the impact that next generation sequencing technology (NGST) is likely to have on clinical practice in this area. The most common form of monogenic SVD is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, due to the mutations in the NOTCH3 gene. Several other inherited forms of SVD include cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy, retinal vasculopathy with cerebral leukodystrophy, collagen type IV α1 and α2 gene-related arteriopathy and FOXC1 deletion related arteriopathy. These monogenic forms of SVD, with overlapping clinical phenotypes, are beginning to provide insights into how the small arteries in the brain can be damaged and some of the mechanisms identified may also be relevant to more common sporadic SVD. Despite the discovery of these disorders, it is often challenging to clinically and radiologically distinguish between syndromes, while screening multiple genes for causative mutations that can be costly and time-consuming. The rapidly falling cost of NGST may allow quicker diagnosis of these rare causes of SVD, and can also identify previously unknown disease-causing variants.

Keywords Stroke · Small vessel disease · Genetics · Next generation sequencing · CADASIL · COL4A1 · CARASIL · Retinal vasculopathy with cerebral leukodystrophy

Introduction

While most strokes are multifactorial in nature, with any genetic contribution likely to result from multiple risk alleles each with small effects, a minority arise due to monogenic causes. These single-gene disorders, usually with high penetrance, may cause stroke as the predominant clinical phenotype, or as part of a systemic disease.

This review covers single-gene disorders which lead to stroke as a major phenotype, with a focus on those which cause cerebral small vessel disease (SVD), an area where there has been significant recent progress with findings that may inform us about the pathogenesis of SVD more broadly. We also discuss the impact that next generation sequencing technology (NGST) is likely to have on clinical practice in this area.

Stroke represents a syndrome which can be caused by multiple pathologies and reflecting this, the majority of monogenic forms of stroke predispose to a single stroke subtype, although a few can present with more than one stroke subtype. Individual examples, presented by stroke subtype, are shown in Table 1.

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Table 1 Summary of monogenic disorders with stroke as a clinical feature

	Ischaemic		Haemorrhagic
Small vessel disease	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)		COL4A1, COL4A2
	Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL)		Hereditary cerebral amyloid angiopathy (CAA)
	Retinal vasculopathy with cerebral leukodystrophy (RVCL)		
	FOXC1		
	COL4A1, COL4A2		
	20q13		
Small and large artery disease	Fabry disease		
	Pseudoxanthoma Elasticum		
	Neurofibromatosis 1		
	Homocystinuria and other genetic causes of hyperhomocysteinemia		
Large artery disease	*Arterial dissection*	*Large artery stenosis*	
	Ehlers-Danlos Type IV	Moyamoya disease	
	Fibromuscular dysplasia	*Atherosclerosis*	
	Marfan's syndrome	Familial	
	Arterial tortuosity syndrome	Hypercholesterolaemia	
Prothrombotic state	Sickle cell disease (also causes intracranial large artery disease)		
	Factor V Leiden		
	Mutations in Prothrombin 2 (F2), Protein S (PROS1), Protein C (PROC), Antithrombin III (AT3) genes		
Embolic stroke	Marfan's syndrome (also arterial dissection)		Fabry disease
	Carney complex (formation of cardiac myxoma)		
	Hereditary haemorrhagic telangiectasia (pulmonary arteriovenous malformations)		
	Hereditary cardiomyopathies		
	Hereditary cardiac dysrhythmias		
Cerebrovascular malformations			*Aneurysms*
			Familial intracranial aneurysm
			Autosomal dominant polycystic kidney disease
			Angiomas
			Cerebral cavernous malformations
Other mechanisms	Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS): metabolic 'stroke-like' episodes not always confined to arterial territories		Fabry disease—due to hypertension arising from renal disease

Monogenic diseases with SVD as a major clinical feature

SVD accounts for about a fifth of strokes, and is the major cause of vascular cognitive impairment and dementia. Radiological features include lacunar infarcts, white matter hyperintensities on T2/FLAIR MRI, asymptomatic cerebral microbleeds on gradient echo MRI, and symptomatic subcortical haemorrhage [1]. Hypertension is the major risk factor but an increasing number of rare monogenic forms are now recognised. There is an overlap between the radiological features and underlying arterial pathology of

ischaemic SVD and subcortical intracerebral haemorrhage (ICH) and some, but not all, monogenic forms of SVD can present with both ischaemic stroke and ICH.

Monogenic SVD presenting with ischaemic stroke

A number of different monogenic diseases present with SVD. Although these have many overlapping features, there are also certain specific features that may help in diagnosis and differentiation (Tables 2 and 3).

CADASIL

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is due to mutations in the NOTCH3 gene [2]. CADASIL is the most common monogenic cause of SVD with an estimated population prevalence in the UK of about 2 per 100000 [3, 4]. A study in 1000 apparently sporadic lacunar stroke patients aged $\leq$70 years found CADASIL mutations in 0.5 %; this was higher (1.5 %) in patients with confluent leukoaraiosis on MRI [5].

Clinical features of CADASIL are confined to the neurological system, the most prominent being migraine, lacunar strokes and cognitive impairment. Migraine, usually with aura, is the first symptom in 60–75 %, with onset usually in the 20s or early 30s. The aura is most commonly visual or sensory but can be dysphasic, and a confusional aura is not uncommon and may merge with the encephalopathic presentation (see below). Strokes are lacunar with a mean age of onset of 46 $\pm$ 9.7 years in one recent study, although first stroke can occur in the 70s [6].

Psychiatric disturbances particularly depression and apathy but also anxiety are common, and onset of depression may precede any other symptoms. Cognitive impairment, with early involvement of selective executive dysfunction and impaired processing speed is common in middle age and may progress to dementia [7]. Occasional cases may present with dementia without symptomatic strokes [8]. Seizures, both focal and generalised, are rare but well described [7].

Patients may experience encephalopathic episodes (also known as a 'CADASIL coma'), a reversible acute confusional or coma often with seizures usually developing from a migraine and lasting as long 7–14 days [6]. These seem to be more likely to occur around the puerperium [9]. Spinal cord involvement is very rare but a few cases have been reported [10].

The earliest radiological features are diffuse T2 white matter hyperintensities up to 15 years prior to the onset of symptoms, with patients typically having an abnormal MRI by age 35 [11]. As symptoms develop, this progresses to confluent leukoaraiosis with characteristic involvement of the anterior temporal pole (Fig. 1). This feature is rare in sporadic SVD but has been shown to have high sensitivity and specificity in diagnosis of CADASIL [12]. Other MRI markers include lacunar infarcts, subcortical microbleeds and enlarged perivascular spaces [13].

CADASIL has distinctive histopathological features on electron microscopy with granular osmiophilic material (GOM) accumulating around the smooth muscle cells of blood vessels. GOM deposition gradually replaces the vascular smooth muscle cells, and occurs in both peripheral and CNS arteries, primarily in the leptomeningeal and small penetrating arteries [14]. Electron microscopy for GOM in skin and muscle biopsies has been used in diagnosis [14].

The severity of the phenotype varies markedly both between and within families, and disease severity does not relate to mutation site [15]. Family based studies suggest other genetic factors may influence disease severity [16]. Both hypertension and smoking have been associated with an earlier age of onset of stroke in CADASIL, while higher blood pressure has been related to more rapid progression of MRI lesion volume [16]. Therefore, aggressive management of risk factors should be implemented [6].

CARASIL

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is caused by autosomal recessive mutations in the HTRA1 gene [17]. It has been described in around 50 individuals in Japanese and Chinese populations [17], and recently cases have been described in European populations [18].

Despite the similarities in name, CARASIL and CADASIL have major phenotypic differences. CARASIL patients, like CADASIL, develop SVD manifesting as recurrent lacunar infarcts (mainly in the basal ganglia or brainstem), progressive cognitive and motor impairment, seizures and psychiatric disturbances including personality changes, emotional lability, abulia and apallic syndrome [17]. However, patients also develop non-neurological symptoms including early-onset diffuse alopecia and degenerative disc disease resulting in acute middle to lower back pain. Patients with CARASIL have a more rapid progression of symptoms, developing dementia and becoming bedridden by around 40 years [17].

On MRI, confluent white matter hyperintensities and lacunar infarcts are seen [19]. White matter hyperintensities often precede symptom onset, and are usually in the deep white matter and periventricular regions, sparing the subcortical arcuate fibres as seen in CADASIL [17]. The involvement of anterior temporal lobes and external capsule has been documented in some patients [20].

Histopathological features of CARASIL are similarly non-specific for the disease, with sporadic SVD-type

Table 2 Monogenic disorders with strokes arising from SVD as a major clinical feature

	CADASIL	CARASIL	RVCL
Disorder	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy	Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy	Retinal vasculopathy with cerebral leukodystrophy
Locus	19p13	10q26	3p21
Gene	NOTCH3	HTRA1	TREX1 (also causes Aicardi-Goutières Syndrome)
Gene product	Notch3 transmembrane receptor	HtrA1 serine peptidase	TREX1 3' exonuclease
Mode of inheritance	Autosomal dominant	Autosomal recessive	Autosomal dominant
Clinical features	Migraine with aura Recurrent subcortical ischaemic strokes Psychiatric disturbances Progressive subcortical cognitive impairment Seizures Encephalopathic episodes Retinal arteriolar narrowing	Recurrent subcortical ischaemic strokes Cognitive impairment Motor impairment Early-onset diffuse alopecia Degenerative disc disease	Visual loss: capillary obliteration, avascular areas in retina, microaneurysms, telangiectatic capillaries Migraines Subcortical ischaemic or haemorrhagic strokes Cognitive impairment Systemic involvement in some patients—hereditary systemic angiopathy
Radiological features	White matter hyperintensities involving external capsules and anterior temporal lobes Lacunes of presumed vascular origin Brain atrophy Cerebral microbleeds	White matter hyperintensities	White matter hyperintensities Subcortical contrast-enhancing mass lesions with surrounding oedema (pseudotumours), mainly in frontal and parietal lobes
Pathological features	Granular Osmiophilic Material (GOM) surrounding vascular smooth muscle cells	Arteriopathy and arteriosclerosis similar to sporadic SVD No GOM	Small blood vessels with fibrinoid necrosis, thickened fibrotic walls, oedematous white matter, prominent reactive astrogliosis—mainly in pons, cerebellum, basal ganglia, fronto-parietal region
Diagnosis	Molecular genetic testing Skin biopsy to demonstrate GOM	Molecular genetic testing	Molecular genetic testing

	FOXC1	COL4A1	COL4A2
Disorder	Axenfeld-Rieger Syndrome, cerebellar malformations	COL4A1-related disorders	COL4A2-related disorders
Locus	6p25	13q34	13q34
Gene	FOXC1	COL4A1	COL4A2
Gene product	Forkhead box transcription factor C1	Alpha-1 chain of collagen IV	Alpha-2 chain of collagen IV
Mode of inheritance	De novo or inherited mutations, reciprocal translocations	Autosomal dominant Autosomal recessive	Autosomal dominant De novo mutations
Clinical features	Subcortical infarcts Axenfeld Rieger Syndrome—retinal arteriolar tortuosity, cataracts, glaucoma, ocular anterior segment dysgenesis, dental, cardiac, umbilical anomalies Cerebellar malformations Hearing impairment	Porencephaly, prenatal bleeding leading to hydroencephaly Infantile hemiparesis Recurrent deep intracerebral haemorrhages TIAs and ischaemic strokes Visual loss Developmental delay, cognitive impairment, dementia Seizures Nephropathy, myopathy, cardiac involvement	Porencephaly Infantile hemiparesis Recurrent deep intracerebral haemorrhages Nephropathy, myopathy

Table 2 continued

	FOXC1	COL4A1	COL4A2
Radiological features	Cerebral small vessel disease which may involve anterior temporal poles and external capsules	Cerebral small vessel disease sparing temporal lobes	Cerebral small vessel disease
	Hydrocephalus	Periventricular cysts involving subcortical structures	Intracranial aneurysms
	Periventricular heterotopia	Deep intracerebral haemorrhages , lacunar ischaemic strokes	
	Cerebellar malformations	Intracranial aneurysms	
		Retinal small vessel abnormalities	
		Distinct phenotype: HANAC syndrome: hematuria, renal cysts, muscle cramps with elevated CK, bilateral, small aneurysms of intracranial segment of ICA	
Pathological features	Not reported	Thickening and focal disruptions of capillary basement membrane	Fragmentation and duplication of epidermal basement membranes
		Dissociated smooth muscle fibres with abnormal spreading of basement membrane in HANAC syndrome	
Diagnosis	Molecular genetic testing	Molecular genetic testing	Molecular genetic testing
Management options	Symptomatic management	Symptomatic management	Symptomatic management
		Caesarean delivery to avoid head trauma	Caesarean delivery to avoid head trauma
		Avoid anticoagulants	Avoid anticoagulants

changes such as arteriopathy (loss of vascular smooth muscle cells) and arteriosclerosis (hyaline degeneration of the media, non-occlusive fibrous intimal thickening of the intima, fragmentation of the internal elastic lamina and narrowing of the vascular wall) of the small vessels. These changes are most apparent in the white matter and basal ganglia, and are found alongside extensive white matter degeneration. No GOM deposits are found [21].

Retinal vasculopathy with cerebral leukodystrophy (RVCL)

Retinal vasculopathy with cerebral leukodystrophy (RVCL) is an autosomal dominant disease of the small vessels caused by mutations in the TREX1 gene [22]. A number of syndromes previously thought to be separate entities are now subsumed under RVCL, having been found to have a common genetic aetiology and overlapping phenotypes: cerebroretinal vasculopathy (CRV), hereditary endotheliopathy, retinopathy, nephropathy and stroke (HERNS) and hereditary vascular retinopathy (HVR) [22].

RVCL typically presents with progressive visual impairment in the 4th to 5th decade, secondary to retinal vasculopathy, neovascularization of the optic disc, retinal haemorrhages, macular oedema microaneurysms, and capillary obliteration starting in the macula. Fluorescein angiograms show telangiectatic capillaries and avascular areas in the retina [23].

Patients later develop neurological features in the form of ischaemic strokes and TIAs, migraine, cognitive impairment, psychiatric abnormalities (such as personality disorders, depression and anxiety) and seizures, with a progressive decline to mortality around 5–10 years after the onset of symptoms [23].

A systemic variant, described as hereditary systemic angiopathy (HSA) or RVCL with multiorgan involvement, has been reported in some families. These patients develop systemic small vessel vasculopathy resulting in the premature infarction and necrosis of tissue, manifesting as Raynaud's phenomenon, hepatic micronodular cirrhosis, renal dysfunction and osteonecrosis [24].

On MRI, there are features of SVD but in addition, contrast-enhancing mass lesions can occur in the deep white matter of the cerebrum and cerebellum Also known as pseudotumours, these masses are surrounded by vasogenic oedema, displacing surrounding structures and regressing in size over several months [25]. Although unique to RVCL, some patients with early-onset cognitive impairment and strokes do not develop these features on imaging [26].

Pathological examination of pseudotumours demonstrates areas of coagulative necrosis secondary to

Table 3 Monogenic disorders with strokes arising from both small and large artery disease as a clinical feature

	Fabry Disease	Pseudoxanthoma elasticum	Neurofibromatosis 1	Homocystinuria
Locus	Xq22	16p13.1	17q11.2	21q22
Gene	GLA (galactosidase alpha)	ABCC6	NF1	CBS (most commonly affected)
Gene product	Alpha galactosidase A enzyme	Multidrug resistance-associated protein 6	Neurofibromin	Cystathionine β synthase
Mode of inheritance	X-linked	Autosomal dominant Autosomal recessive	Autosomal dominant	Autosomal recessive
Key clinical features	Small fibre peripheral neuropathy with acute pain crises Ischaemic and haemorrhagic strokes Progressive renal failure Angiokeratomas Tortuous retinal vessels, whorl keratopathy on slit-lamp exam Cardiomyopathy, hypertension	Cardiovascular: hypertension, angina, intermittent claudication, restrictive cardiomyopathy, mitral valve prolapse, bleeding in GIT Ischaemic stroke Skin lesions: yellow papules Ocular signs: visual impairment, peau d'orange, angioid streaks on retina, neovascularization, retinal haemorrhages.	Skin: neurofibromas, café-au-lait spots, freckling Eyes: Lisch nodules PNS: neurofibromas, schwannomas Meninges: dural ectasia Skeleton: scoliosis, dysplasia Endocrine: phaeochromocytoma Strokes	Developmental delay and intellectual disability Arterial and venous thrombotic disease affecting small and large vessels e.g. paediatric strokes Dislocation of optic lenses, severe myopia Skeletal abnormalities or osteoporosis
Radiological features	Large infarctions Cerebral SVD Dolichoectasia of intracranial arteries	Deep white matter lesions Lacunar infarcts	Collateral circulation (Moyamoya appearance) due to occlusive lesions	Cerebral SVD Intracranial artery occlusion Evidence of strokes including venous sinus thrombosis
Pathological features	Kidney biopsy: lipid staining may show Gb3 lysosomal inclusions in whorled layers of alternating dense and pale material ('zebra bodies')	Calcified, fragmented elastic fibres in skin, eyes, arteries	Proliferation and thinning of media resulting in luminal stenosis Aneurysmal intimal changes Nodular periarterial changes Epitheloid changes	Fibrous arteriosclerotic plaques
Diagnosis	Clinical: angiokeratomas, renal disease, painful neuropathy, whorl keratopathy on slit-lamp Biochemical: low α-galactosidase in plasma and peripheral leukocytes Genetic testing in females with low-normal α-galactosidase	Molecular genetic testing Skin biopsy not diagnostic	Molecular genetic testing	Plasma homocysteine >100micromol/l Blood and urine amino acid profiles Direct enzyme assays Molecular genetic testing
Management options	α-galactosidase A replacement Analgesics, antiarrhythmics, dialysis, renal transplant	Prophylactic measures—avoid contact sports	Surgery or chemotherapy for lesions	Folic acid ± vitamin B6, B12 supplementation

obliterative vasculopathy [22]. These areas are surrounded by oedematous white matter with prominent reactive astrogliosis similar to that seen in radiation necrosis. Small vessels are often occluded by fibrin thrombi, with thickened fibrotic walls and a distinctive multilamellar subendothelial basement membrane [22].

COL4A1/A2 related arteriopathy

A spectrum of conditions with both infantile and adult onset, and neurological and systemic features, has previously been described as four separate conditions: autosomal dominant Type I Porencephaly, brain SVD with

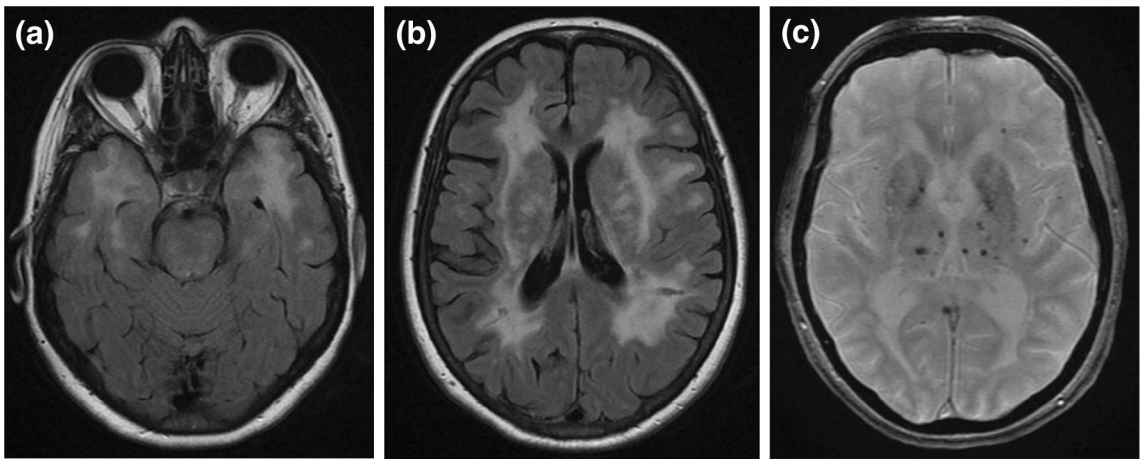

Fig. 1 MRI appearances of CADASIL. White matter hyperintensities involving the anterior temporal pole (**a**) and external capsule (**b**) are seen on T2/FLAIR MRI sequences and subcortical cerebral microbleeds can be seen in some patients are seen on T2* Gradient-Recalled Echo (T2*GRE) sequences

haemorrhage, brain SVD with Axenfeld-Rieger Anomaly and Hereditary Angiopathy with Nephropathy, Aneurysms and muscle Cramps (HANAC syndrome). These were later found to be attributable to the same genotype: autosomal dominant mutations in the COL4A1 gene which encodes the Type IV collagen α1 chain [27].

COL4A1 arteriopathy can present in childhood with porencephaly, infantile hemiparesis and developmental delay, and children may develop intracerebral haemorrhage sometimes associated with trauma and anticoagulation [28]. In a mouse model with COL4A1 mutations, intracerebral haemorrhage occurred during birth, and was avoided by caesarean section, lending support to the association found between haemorrhage and head trauma [29]. More recently it has been recognised that carriers may present for the first time in adulthood with intracerebral haemorrhage, usually subcortical, and ischaemic lacunar infarcts. Such patients often have white matter hyperintensities on MRI and microbleeds on T2* sequences are very common [28].

Other neurological features include seizures, cognitive impairment and dementia and visual loss. Patients with visual loss also show marked tortuosity of the medium and small retinal arterioles and venules, alongside retinal ischaemic changes [30]. Systemic involvement can be a feature, with renal agenesis, muscle and cardiac symptoms, as well as Raynaud's syndrome [27]. The phenotypes seen in COL4A1 angiopathy often vary widely and the phenotype and age of onset can vary greatly between individual family members [27].

On neuroimaging, patients with the porencephalic phenotype demonstrate fluid-filled periventricular cysts which involve subcortical structures in the brain [28]. Intracranial aneurysms may be seen usually in the intracranial portions of the internal carotid artery [28]. White matter hyperintensities are found mainly in the supratentorial posterior periventricular, frontal and parietal areas, sparing arcuate fibres. Unlike CADASIL, these also spare the temporal lobes. Dilated perivascular spaces are mainly seen in the basal ganglia, while microbleeds are prominent and found both in the subcortical region and cortical-subcortical junction (Fig. 2) [28].

Histopathological examination reveals thickening and focal disruptions of capillary basement membranes, and dissociated smooth muscle fibres with abnormal spreading of the basement membrane at the dermo-epithelial junction and kidney tubules [31].

Mutations in the COL4A2 gene at the same chromosomal locus have also been found to cause a similar phenotype of cerebral SVD manifesting as recurrent intracerebral haemorrhage, early-onset porencephaly, congenital hemiplegia, intracranial aneurysms, nephropathy and myopathy [32]. Histopathological features are similar to that seen in COL4A1, with thickening and duplications of the basement membrane on electron microscopy of the skin [33].

Fabry disease

Fabry disease is an X-linked metabolic disorder caused by mutations in the GLA gene on chromosome Xq22, which encodes the lysosomal enzyme, α-galactosidase A. This results in the defective metabolism of globotriaosylceramide (Gb3), resulting in the accumulation of Gb3 in lysosomes of vascular endothelial cells and smooth muscle cells in multiple organ systems [34].

Hemizygous males tend to first develop small fibre peripheral neuropathy in early childhood. This manifests as acroparesthesia, a burning sensation in a glove-and-stocking distribution, with abnormal sensation of cold in the feet [35]. Patients may also experience excruciating pain crises

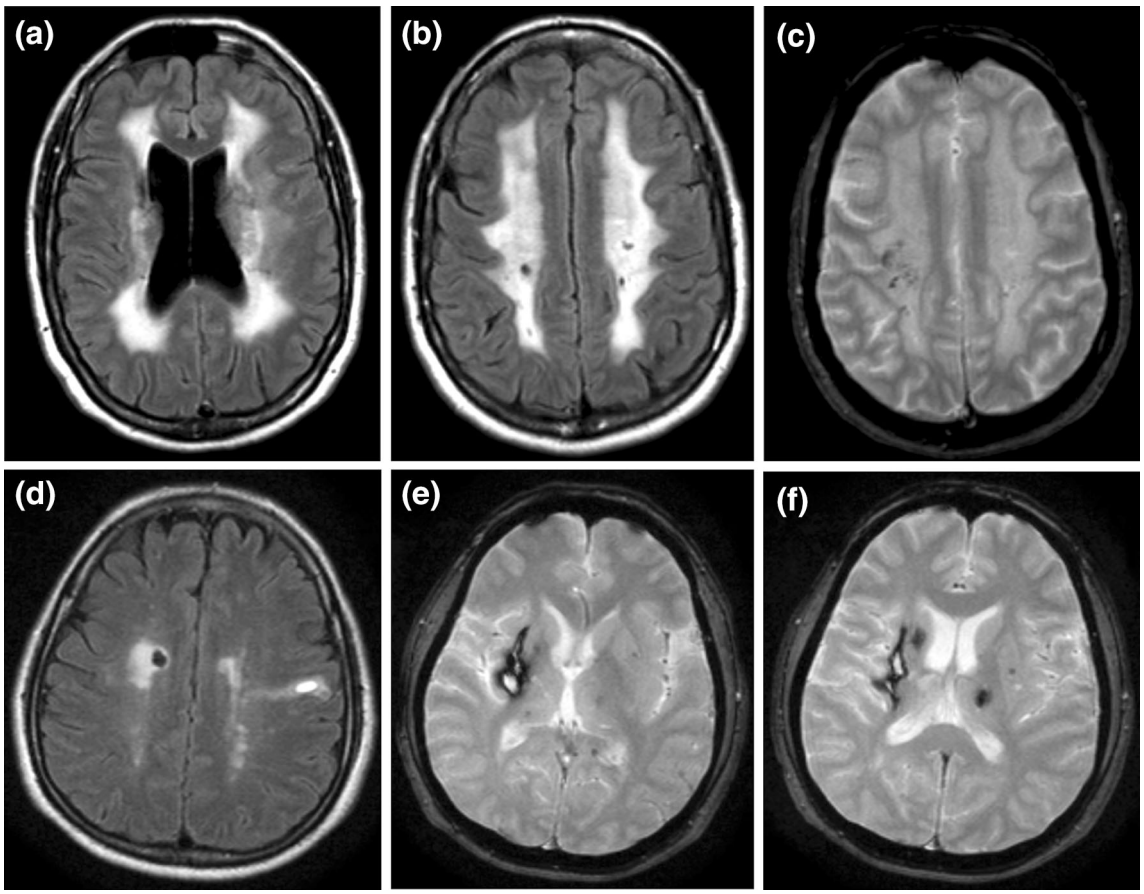

Fig. 2 MRI appearances in 2 patients with COL4A1 mutations. T2/FLAIR sequences showing confluent white matter hyperintensities (**a**, **b**, **d**). T2*GRE sequences show cortical-subcortical microbleeds (**c**, **f**) and a previous haemorrhage (**e**,**f**)

in the hands and feet, precipitated by exercise, fever or hot weather, and generalized anhidrosis [35]. Fabry causes progressive renal failure in the third to fifth decade of life [35]. Patients may also have gastrointestinal, cardiac, respiratory and orthopaedic involvement, with death often resulting from renal, cardiac or cerebral complications [35]. Other systemic features of Fabry disease include asymptomatic ocular involvement, with tortuous retinal and conjunctival vessels, corneal and lenticular opacities and whorl keratopathy seen on slit-lamp examination. They may demonstrate reddish-purple angiokeratomas appearing on the skin, first in the periumbilical area, then on the extensor surfaces of the elbows, knees, hip and genital areas, and mucosal areas such as the mouth [36].

Ischaemic or haemorrhagic strokes arise in the third or fourth decade of life, and can be the presenting feature leading to the diagnosis. These strokes may be due to small vessel or large vessel disease, or secondary to cardioembolism from Fabry related cardiac disease [37]. Large vessel ischaemic events tend to occur in the posterior circulation territory where dolichoectasia of the vertebral arteries can be a feature [37]. White matter hyperintensities are a common feature [38].

An early study in German patients aged 18 to 55 reported that Fabry disease was the cause of 4.9 % of cryptogenic ischaemic or haemorrhagic strokes in males, and 2.4 % in females [39]. Subsequent studies have found lower incidences ranging from 0 to 2 % in younger onset patients with cryptogenic ischaemic stroke [40]. A large study in the UK, with genotyping in 994 men and women with lacunar stroke with onset $\leq$70 years found no classical pathogenic Fabry mutations [5].

Although Fabry disease is an X-linked disease, heterozygous females are not always asymptomatic carriers or have milder symptoms. Females may still be severely affected with multisystem disease and life-threatening manifestations [41]. Hence, while the diagnosis in males can be made biochemically with a low level of α-galactosidase activity in the plasma, affected females may have normal levels of enzyme and require genetic testing for diagnosis [42].

Enzyme replacement therapy has been shown to reduce pain and improve renal function, by clearing endovascular deposits of globotriasylceramide, but whether it reduces future stroke risk is unknown [43].

FOXC1-deletion related SVD

The forkhead box transcription factor 1 (FOXC1) gene on chromosome 6p25 encodes a member of the winged/helix forkhead family of transcription factors. FOXC1 is involved in processes of vascular development, such as in arterial specification and angiogenesis regulation [44]. FOXC1 was initially thought to be the causative gene behind Axenfeld-Rieger Syndrome (ARS) and cerebellar malformations in patients with chromosome 6p25 copy number variations [45]. Patients with ARS present with ocular abnormalities such as anterior segment dysgenesis and early-onset glaucoma, as well as non-ocular features such as systemic dysmorphisms, and dental and umbilical abnormalities [45]. This often overlaps with another phenotype known as the Dandy Walker malformation, characterised by cerebellar vermis hypoplasia and mega cisterna magna [46].

In multiple case reports of patients with 6p25 deletions, individuals with ARS and other developmental abnormalities were found to also have white matter hyperintensities on MRI from as early as 18 months [47]. These reports provided early evidence for involvement of the FOXC1 gene in SVD. A meta-analysis and study of expression quantitative trait loci in GWAS data from the cohort for Heart and Ageing Research in Genomic Epidemiology (CHARGE) consortium later showed that 3 SNPs associated with white matter hyperintensities strongly influenced FOXC1 transcript levels. 18 out of 18 patients with FOXC1-related ARS in the CHARGE study also showed evidence of SVD on MRI. Experimental overexpression and suppression of the FOXC1 gene in zebrafish also led to cerebral haemorrhage, lending further support for the gene's independent involvement in cerebrovascular disease [48].

Monogenic SVD presenting with intracerebral haemorrhage

Both COL4A1/2 (see above) and cerebral amyloid angiopathy (CAA) can present with intracerebral haemorrhage (ICH).

Hereditary cerebral amyloid angiopathy

CAA is a term used to describe a number of conditions involving the deposition of amyloid fibrils in small and medium sized blood vessel walls, and sometimes in the capillaries of the brain parenchyma and leptomeninges [49]. CAA has both hereditary and sporadic forms with similar clinical features, with an earlier age of onset in the hereditary form [49].

Hereditary CAA is usually autosomal dominant, and the majority of cases are due to the deposition of amyloid beta (Aβ), derived from the amyloid precursor protein (APP), encoded by a gene on chromosome 21 [50]. Aβ-type hereditary CAA may also co-occur in familial Alzheimer's disease, with mutations in APP, PSEN1 (Presenilin1) and PSEN2 (Presenilin2) genes [50]. There are also a number of non-Aβ hereditary CAAs [51].

Amyloid is formed when soluble amyloidogenic peptides convert from random alpha-coils to beta-pleated sheet-rich conformations. It appears as apple-green birefringence in Congo red preparations under polarised light. These structures aggregate to form deposits around the smooth muscle cells of vasculature, gradually infiltrating the intima and replacing the smooth muscle cells [49]. The resulting blood vessel walls are fragile, leading to micro and macrohaemorrhages. These damaged blood vessels are unable to autoregulate cerebral blood flow, resulting in chronic ischaemia (resulting in white matter changes) and acute ischaemia in the form of infarcts [51].

Different forms of hereditary CAA present with overlapping clinical features. A common feature of most hereditary CAAs is SVD resulting in small cortical infarcts and recurrent lobar intracerebral haemorrhages, which is characteristic of Aβ forms and HCHWA-Icelandic type CAA. Patients also tend to develop cognitive decline progressing to dementia [51].

On imaging, CAA patients demonstrate lobar microbleeds, extensive small vessel disease, superficial siderosis (haemosiderin deposits in the subpial layer arising from bleeding of a subarachnoid origin) and subcortical infarcts [52]. Cerebral microbleeds can be detected on T2*-weighted Gradient-Recalled Echo (T2*-GRE) MRI sequences [53]. Diagnoses of probable or possible CAA can be made using the modified Boston criteria, which take into account clinical, pathological and radiological features [52]. A definitive diagnosis can only be reached on post-mortem examination or brain biopsy.

It has been suggested that PET imaging using amyloid-specific radioligands, most commonly ^{11}C Pittsburgh Compound B (^{11}C-PiB), which binds to both parenchymal and vascular Aβ in the brain, may be a means to detect CAA in life [54]. ^{11}C-PiB -PET has a high sensitivity of 91 %, and thus has utility in ruling out CAA but a lower specificity [55].

What can we learn from monogenic SVD about disease mechanisms?

The many different genes causing Mendelian forms of SVD demonstrates that multiple different molecular pathologies can result in a similar clinical and radiological phenotype. Discovering unifying pathways may provide novel insights into sporadic SVD which could point the way to new treatment options. Furthermore, it has been

suggested that shared molecular mechanisms could underlie both monogenic and sporadic SVD, and some support for this hypothesis is emerging from studies looking at associations between common polymorphism in genes causing monogenic SVD and sporadic SVD. Common variants in COL4A1/A2 were associated with a spectrum of SVD phenotypes including lacunar stroke, WMH and subcortical ICH [56]. Common Notch3 polymorphisms were associated with an increased risk of age- and hypertension-related white matter lesions [57], although this finding could not be replicated in younger onset lacunar stroke [5].

The role of TGF-β in arteriopathy

The HTRA1 gene codes for high temperature requirement serine peptidase A 1 (HtrA1), a member of a family of oligomeric serine proteases. HtrA1 is a serine peptidase which suppresses signalling by members of the transforming growth factor β family [58]. CARASIL mutations result in the loss or reduction of HtrA1 function, as seen by the increased levels of TGFβ seen in the media of small arteries, and increased TGFβ signalling in CARASIL patients [59]. This contributes to a fibrotic process in the vasculature, possibly leading to reduced cerebral blood flow [17]. The role of TGF-β signalling in fibrotic events of organs has previously been described [60]. Hence, the similarity with the fibrotic changes seen in CARASIL lends support to the involvement of TGFβ signalling in SVD.

Impaired localization of DNA repair molecules

The pathogenesis of RVCL suggests that impaired DNA-damage repair can be a mechanism leading to stroke. The TREX1 gene encodes the most abundant DNA exonuclease in the mammalian cell, DNAse III or Three prime Repair Exonuclease [22]. DNAse III is thought to have roles in DNA repair, as it is normally localised in the endoplasmic reticulum, but is translocated to the nucleus following oxidative DNA insult [24]. RVCL is caused by frameshift mutations in the carboxyl-terminus of the gene, resulting in the expression of DNAse III with a truncated C-terminus which has functions in the localisation of DNAse III in the endoplasmic reticulum. Functional analyses have shown that these mutations cause cellular mislocalization [24], rather than a loss of enzymatic activity.

Loss of vessel wall integrity

Type IV collagen is an integral component for basement membranes, and contributes to the tensile strength of all tissues including vasculature. Collagen also has roles in maintaining vascular tone and endothelial cell function [29]. COL4A1 and COL4A2-related arteriopathy is caused by missense mutations, often resulting in the substitution of a highly conserved hydrophobic glycine residue within the collagen molecule [61]. This alters the three-dimensional conformation of the chain, inhibiting the formation and deposition of a collagen heterotrimer in the vascular basement membrane, resulting in increased vessel wall fragility [29]. Increased blood brain barrier (BBB) permeability is associated with increasing age, and is thought to be a contributing factor to sporadic small vessel disease [62]. The mechanism of stroke in COL4A1 and COL4A2-related arteriopathy, combined with the finding that more common variants in COL4A1 are associated with sporadic SVD [56], thus providing further evidence for the role of BBB compromise, whether due to genetics or ageing, in sporadic SVD.

Lessons from CADASIL: common Events and pathways?

Recent developments in our understanding of CADASIL pathogenesis suggest a possible unifying disease pathway between CADASIL, CARASIL and COL4A1/COL4A2-related arteriopathies, as well as other neurodegenerative diseases.

CADASIL is caused by mutations within the NOTCH3 gene, which encodes a transmembrane receptor which is primarily expressed on vascular smooth muscle cells and pericytes in the brain. The physiological proteolysis of NOTCH3 produces a 210 kDa extracellular fragment (ectodomain) and 97 kDa intracellular fragment, which form a heterodimer at the vascular smooth muscle cell plasma membrane. In response to Delta/Jagged ligand binding, the intracellular domain translocates to the nucleus and activates the transcription of genes via the RBPJ pathway [63].

CADASIL-causing mutations arise within the extracellular domain of the receptor, which contains 34 epidermal growth factor-like repeats. Almost all mutations result in an odd number of cysteine residues [64].

Much data suggest the disease is not due to a loss of function of the NOTCH3 receptor, at least in the vast majority of cases, as patients with hypomorphic NOTCH3 alleles do not show signs of CADASIL [65]. Furthermore, $NOTCH3^{-/-}$ mice do not show GOM in their vasculature, in comparison to transgenic NOTCH3 mice carrying CADASIL-causing mutations which demonstrate early NOTCH3 ectodomain and GOM accumulations, as well as white matter lesions [66]. An early feature is accumulation of NOTCH3 ectodomain-containing aggregates in the tunica media of small vessels, in close proximity to GOM accumulations around the smooth muscle cells [67]. Recent studies in vitro have demonstrated the link between

mutations and multimerization of the NOTCH3 ectodomain, providing evidence for the direct pathogenic effects of the mutations [68].

Two other proteins have been found to be components of GOM: clusterin, an extracellular chaperone that has been implicated in the formation of extracellular deposits in Alzheimer's disease and other disorders, and endostatin, a proteolytic fragment derived from collagen 18 alpha-1 (COL18A1), which has anti-angiogenic properties [69].

Proteins which aggregate together with the NOTCH3 ectodomain have also been identified. Using mass spectrometry, two other proteins, TIMP3 (tissue inhibitor of metalloproteinase 3) and VTN (vitronectin), were found in the blood vessels of patients and mutant transgenic mice. On pathological examination, these were found to be present within the NOTCH3 ectodomain aggregates, and co-immunoprecipitation showed that increased levels of aggregation of NOTCH3 ectodomain promote complex formation with TIMP3, and TIMP3 promotes aggregation of NOTCH3 and VTN [70].

The NOTCH3 ectodomain accumulation may serve as a 'seed' to promote further protein aggregation, in a fashion similar to that seen in neurodegenerative diseases where there is prion-like propagation of protein aggregates [71]. In fact, the study of TIMP3 in CADASIL has also revealed other similarities with neurodegenerative disease—an increase in TIMP3 protein levels and activity that did not correlate with mRNA levels, suggesting that there was either an increased translational efficiency or impaired protein degradation leading to TIMP3 accumulation [70]. This is reminiscent of other neurodegenerative proteopathies including Parkinson's disease, Alzheimer's disease and CAA, where an altered balance between production and clearance of proteins have been shown to also underlie the formation of protein aggregates [71].

TIMP3 has multiple functions, including anti-angiogenic and matrix metalloproteinase inhibitory activity, while VTN regulates fibrinolysis. Hence, both TIMP3 and VTN have roles in maintenance of the extracellular matrix, and abnormal activity of these proteins can result in vessel fibrosis [70]. This suggests a possible link between CADASIL and COL4A1-related arteriopathy, where the impaired secretion of Collagen IV for the formation of the basement membrane results in SVD.

Other proteins found to aggregate with the NOTCH3 extracellular domain, such as thrombospondin-2, are also known to play a key role in extracellular matrix regulation [68]. In a pathological study, a member of the TGFβ-binding protein (LTBP) family, LTBP-1, was found to co-localize with NOTCH3-ectodomain aggregates, and to promote the accumulation of latency-associated peptide (LAP) in the aggregates [72]. LTBP members are components of the extracellular matrix which have a role in TGFβ activation. In particular, LTBP-1 sequesters latent TGFβ within the extracellular matrix through covalent interaction with latency-associated peptide (LAP) [72].

The involvement of LTBP-1 in CADASIL aggregates suggests that the disruption of normal TGFβ signalling may not only be involved in CARASIL, but also have functions in CADASIL pathogenesis. The role of the extracellular matrix in TGFβ activation also brings into question whether the disruption of the basement membrane in COL4A1/COL4A2-related arteriopathies is also an early event in this common pathway involving TGFβ.

The discovery of other proteins and pathways in CADASIL that are also implicated in CARASIL and COL4A1/COL4A2-related arteriopathies indicates that the different disease pathways intersect at a common pathway culminating in SVD. It is possible that TGFβ and extracellular matrix integrity both play integral roles in the pathogenesis of SVD, and that the relative importance of each pathway, depending on the responsible mutation, determines which disease manifests.

A schema linking possible mechanisms by which the different monogenic forms of SVD might cause small vessel arteriopathy is shown in Fig. 3.

Diagnosing monogenic forms of SVD

Key features suggesting a monogenic form of SVD are a family history and early age of onset (Box 1). It is important to take a structured history asking individually about individual family members, and remembering that SVD may have been misdiagnosed as other neurological disease; common examples being vascular dementia as Alzheimer's dementia and ischaemic white matter changes in CADASIL as multiple sclerosis. There may be additional clues present in the history, on examination or on neuroimaging (see Tables 2 and 3).

Sometimes a diagnosis can be strongly suspected on clinical grounds, for example, a clinical phenotype compatible with CADASIL and characteristic anterior temporal pole involvement on MRI. However, this is often not the case and it can be difficult to decide in which cases one should progress to molecular genetic testing. Current mutation screening tests one gene at a time and screening individual genes is expensive. The decision of which candidate gene to test for mutations in is not often straightforward, due to overlapping phenotypes between each disorder and the heterogeneity of phenotypes within families. Furthermore, some tests may not be easily accessible, while patients have been described with an apparently monogenic SVD disorder but have no mutations in known genes. The increasing use of next generation sequencing technology (NGST) in clinical diagnosis may make major contributions in these areas.

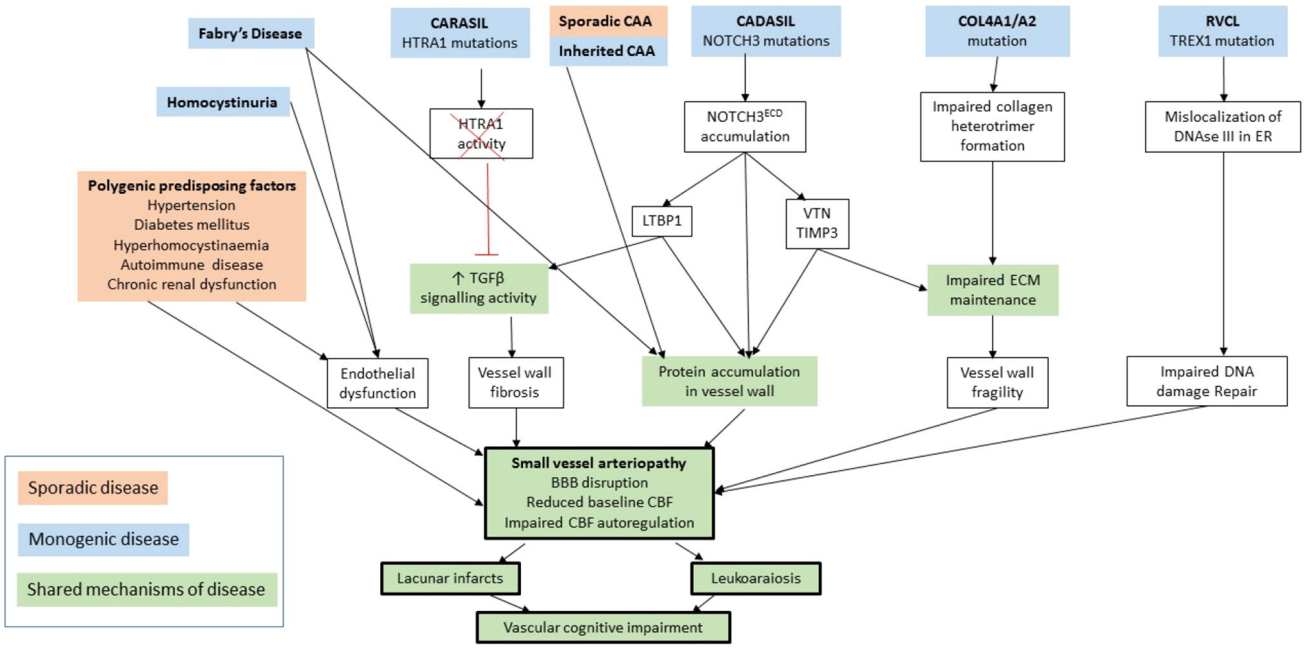

Fig. 3 Mechanisms in monogenic forms of small vessel disease, and possible shared mechanisms with sporadic SVD

Next generation sequencing and its impact on SVD

Next generation sequencing technologies (NGST) refers to highly parallel DNA sequencing technologies that can produce many hundreds of thousands, or millions of short reads (25–500 bp) for a low cost and in a short time. These technologies can deliver complete genome sequences (whole genome sequencing, WGS), or the coding sequences only (whole exome sequencing, WES), which covers about 1 % or 30 million base pairs of the genome. WES techniques involve the targeted resequencing of protein-coding DNA sequences by using hybridization techniques to capture these sequences [73]. Other forms of NGST include sub-exome panels of specific genes, RNA sequencing and methylation sequencing.

These high-throughput techniques were initially used to provide further information (by targeted resequencing) on susceptibility loci identified by GWAS. With a growing understanding of rare disease-causing variants, NGS is now moving from being a predominantly research tool to a diagnostic tool, and may ultimately become routine assessment for patients suspected of having a rare inherited disorder. These studies rely on comparisons between unaffected and affected related individuals, or between affected unrelated individuals, to discover novel alleles [74].

NGS is likely to impact on management and understanding of SVD in a number of ways. First, it may aid identification of additional monogenic forms of SVD. Second, it is likely to be increasingly used in clinical diagnosis.

Despite the recent discovery and characterization of monogenic causes of stroke, such as RVCL and COL4A1, there still exist undiscovered inherited forms of the disease which may present with similar symptoms to known diseases. For example, a Swedish kindred, presented with stroke-like episodes, neuropsychiatric symptoms and progressive dementia, with white matter hyperintensities not affecting the anterior temporal lobes or external capsules. This family was suspected of having CADASIL, but NOTCH3 sequencing showed no pathogenic mutations and skin biopsy showed no GOM. Linkage analysis showed no distinctive haplotype attributed to the affected family members [75]. Since then, a CADASIL-like syndrome not due to NOTCH3 mutations has been described, where clinical features are largely similar to CADASIL apart from a later age of onset of stroke and a later age of onset in family members [76].

NGS offers the opportunity to identify novel causative genes underlying these disorders, and an increasing number of new monogenic causes of neurological disease have been recently reported [77].

NGS is likely to have a major impact on clinical testing for monogenic SVD. Testing multiple individuals' genes is costly and time-consuming and NGS offers the opportunity to exclude all known causes in a single assay. NGS approaches include both small targeted gene panels and whole exome and whole genes sequencing. Targeted gene panels are already routinely used in the clinical setting, such as for hereditary colon cancer, cardiovascular and neurodegenerative disease [78], and this approach is now

being developed for monogenic SVD. Whole genome approaches are increasingly used and their widespread clinical application in diagnosis is being evaluated in a number of large scale studies, including the Genomics England project [79].

NGST in the clinic: what are the challenges?

Interpretation of sequencing results

The greatest challenge in the use of NGST for diagnosis is the interpretation of the large amount of data produced. As the functions of most genes are still poorly understood, it is difficult to determine which gene variant is implicated in the disease, and if the disease is truly caused by the identified variant. These variants of uncertain clinical significance (VUCS) may be erroneously filtered out if they are present in control populations. Public databases may not be a representation of what is normal, but only a reflection of what has been sequenced. Filtering is often based on the assumption that the filter set contains no alleles from the affected individuals; however, it is more likely that there are a small number of pathogenic alleles that do segregate into the general population with low frequencies. In recessive disorders where the carrier status does not result in disease, carriers may thus be erroneously included in the control set [80]. The presence of variants in the same gene in other patients with the same disease may confirm the new pathogenic variants, but further functional experiments are usually necessary to validate the role of these variants.

Despite the relative homogeneity of phenotypes in monogenic disease when compared to sporadic disease, genotype–phenotype correlation is also a challenge. This is due to the wide variation in clinical phenotypes and incomplete penetrance of some genes. Filtering the thousands of apparently novel mutations detected using family data and databases, may narrow down variants of interest. However, even with intensive filtering, there are likely to be pleiotropic variants (where the same mutation in the same gene may have different phenotypic effects). For example, hexanucleotide intronic expansions in C9ORF72 have been found to lead to the presence of TDP43 positive inclusions in the CNS in both frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Yet, despite this, similar genotype and pathology, the clinical presentations of ALS and FTD are vastly different [81]. This lack of one gene–one disease correlation can pose a significant challenge to identifying a single causative variant.

Sequence coverage

This is an important issue for the use of NGS in clinical diagnosis. Despite the advances in sequencing technology, sequence coverage remains a limitation. Coverage may be impaired by repetitive genomic regions, long insertion-deletion variations, structural variants and aneuploidy.

False negative rates may vary according to genomic regions, and probes often do not sequence certain genes, either because it was not selected during the assay development stage, or due to the presence of repetitive sequences.

Poorly performing probes due to GC-rich sequences or low mapping quality may exist. Hence, NGST results may need to be validated using other existing technology, such as Sanger sequencing, long range PCR amplification and copy number detection approaches [78].

Conclusion

Monogenic diseases are a rare but important cause of stroke. Although rare, making a diagnosis is important for the individual patient, and allows predictive testing of other family members, as well as the possibility of prenatal testing. Furthermore, the characterization of these diseases has provided us with experimental models of sporadic disease, and contributed to our understanding of possible common mechanisms behind SVD.

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Ethical standard The manuscript does not contain clinical studies or patient data.

Box 1: Features that heighten clinical suspicion of a monogenic cause of SVD. Note many of these are indicators but not diagnostic. For example, CADASIL can occur in patients with risk factors which may indeed exacerbate the phenotype

Clinical presentation

- Onset of stroke at an early age.
- Syndromic disease: history of other clinical features which fit with recognised monogenic stroke syndrome:
  - Other neurological history such as complicated migraines, seizures, early-onset cognitive impairment, psychiatric disturbances.
  - Non-neurological features such as skeletal, facial, ocular abnormalities.

Risk factors and other causes of white matter disease

- The absence of identifiable risk factors such as diabetes, hypertension or smoking.
- The absence of any other cause of stroke.

Family history

- A family history of early-onset stroke or dementia, especially if this is occurring in a Mendelian pattern of inheritance.

Presence of atypical features of imaging, such as

- Evidence of SVD beyond what is expected for age and risk factors.
- Atypical distribution of white matter hyperintensities on T2/FLAIR MRI in anterior temporal poles and external capsule as seen in CADASIL.
- Extensive microbleeds particularly in COL4A1/2 mutations.
- Pseudotumours as seen in RVCL.
- Vascular malformations such as aneurysms (COL4A1), dolichoectasia (Fabry Disease).

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