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Genetic and environmental associations with pediatric cerebral arteriopathy: insights into disease mechanisms

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Introduction

Mechanisms underlying childhood arterial ischaemic stroke (AIS) are heterogenous and poorly understood but critical for the development of targeted interventions. Current evidence suggests interplay between (mostly) rare genetic risk factors and common environmental exposures. Here we explore this interplay in relation to cerebral arteriopathies associated with childhood AIS.

Background

Most childhood AIS is associated with non-atherosclerotic cerebral arteriopathy(1, 2) - a term encompassing any pathologic abnormality of the arterial circulation. This non-specific terminology reflects paucity of knowledge regarding underlying mechanisms. In 2014, the Vascular Effects of Infection in Pediatric Stroke (VIPS) study defined arteriopathy as "the imaging appearance of an *in situ* arterial abnormality (stenosis, irregularity, occlusion, banding, pseudoaneurysm, dissection flap) not attributable to an exogenous thrombus (e.g., cardioembolism) and not considered a normal developmental variant."(3) It further defined arteriopathy subtypes using the 2004 consensus-based definitions described by Sébire et al and the 2012 CASCADE criteria.(4,5) These definitions use radiological and clinical characteristics to categorise patients; however, biomarkers separating distinctive arteriopathy subtypes are still lacking.

Arteries are maintained by dynamic processes responsive to genetic and local signals. Congenital arteriopathies could reflect abnormal arterial development, while acquired arteriopathy could arise from disruptions in "vascular homeostasis" e.g. endothelial injury, repair, angiogenesis.(6)

Overall, arteriopathy increases AIS recurrence risk, that varies with subtype.(7) Diagnosis can be challenging – even with rigorous review, arteriopathy diagnosis could not be adjudicated in 34/355 cases.(3) Careful phenotyping is important; e.g. a pattern initially called moyamoya (MM) had distinctive features in *ACTA2* mutations (figure 1).(8) It is not meaningful to consider all arteriopathies or AIS as a single group as they likely have different aetiologies, disease course and treatments.

Environmental associations with childhood AIS include infection and trauma. Clinically trivial viral infection and minor trauma are ubiquitous in childhood, yet AIS is rare - so additional factors, such as genetic predisposition, are likely to be important. Inflammation is likely to play a variety of roles in childhood AIS subtypes. Though monogenic causes of childhood AIS are rare, single gene associations with arteriopathy provide important mechanistic insights. Increasingly it appears that, in many of these, interaction between genetic predisposition and environmental factors is necessary to produce the ultimate disease phenotype.

Genetic association with childhood cerebral arteriopathy

Table 1 and supplementary table I summarise single gene mutations associated with pediatric cerebral arteriopathy and highlight concurrent phenotypic features.

Moyamoya (MM)

MM is a rare, severe and often progressive cerebral arteriopathy, defined radiologically by occlusive disease of the terminal internal carotid arter(ies) with basal collaterals.(9) MM is divided into MM disease (MMD - primary or isolated), and MM syndrome (MMS; secondary to another disease, often genetic). Children with MM are at risk of AIS, while adults are more prone to hemorrhagic stroke. MM is arguably the most malignant pediatric cerebral arteriopathy phenotype, with highest risk of AIS recurrence.(7, 10)

The strong ethnic bias (in East Asians) and 15% familial cases strongly implicate a genetic basis. Linkage studies have identified several HLA alleles associated with MMD in East Asians: HLA-B35, HLA-B51, HLADRB1*0405, DQB1*0502, and *0401. MMS is also seen in Trisomy 21, Turner's syndrome, sickle cell disease, neurofibromatosis type 1 (NF1); other associations are summarised in table 1.

The p.R4810K in *RNF213* polymorphism is associated with MMD in Japanese and Koreans.(11, 12) As Japanese prevalence of MMD is 6/100,000 , compared with 1% carrier rate of p.R4810K, additional factors must be required to produce MM in carriers.(12, 13) *RNF213* p.R4810K polymorphisms have been reported in small numbers of East Asian patients with other genetic diagnoses which predispose to MMS, including NF1 and trisomy 21; thus *RNF213* may act as an additional modifier.(14, 15) Evidence from *RNF213* knockouts suggests that loss of function could result in vascular fragility, and susceptibility to haemodynamic stress and other insults.(16) Additional postulated mechanisms include a pro-inflammatory effect resulting in endothelial cell dysfunction, and proliferation of smooth muscle cells with vascular stenosis.(17)

Neurofibromatosis type 1 (NF1)

NF1, an autosomal dominant tumour-suppressor syndrome caused by mutations in the *NF1* gene, is associated with MMS and other large-vessel arteriopathies (aneurysm and arterial stenosis).(18, 19) *NF1* encodes for neurofibromin, a Ras pathway inhibitor. Children with NF1 have an increased risk of stroke, with odds ratios of 8.1 for hemorrhagic stroke and 3.4 for AIS.(20) Radiotherapy for optic glioma is an additional risk factor, but even discounting this cerebral arteriopathy has been reported in 2.5% - 6% of NF1 patients.(21) Patients with asymptomatic MMS may show radiological progression and could benefit from antiplatelet therapy or revascularisation.(22)

Neurofibromin is expressed in vascular endothelial cells, is a regulator of macrophage function, and is likely to have a role in pathogenesis. NF1 patients without arteriopathy have elevated levels of inflammatory cells and cytokines linked to vascular disease; thus, they may be susceptible to vascular disease, but a second "hit" may be required to generate arteriopathy.(23)

Most cases of MMS in NF1 are reported in Caucasians, and this also implicates additional genetic predisposition to MMS in NF1. *RNF213* mutations have been reported in a small group of Korean patients with NF1 and MMS, and this may be a gene modifier.(14)

Mutations causing smooth muscle cell dysfunction

ACTA2

Alpha-actin (encoded by *ACTA2*) forms a major part of the vascular smooth muscle cell contractile apparatus. Heterozygous *ACTA2* mutation carriers are at risk of vascular disorders, including aortic aneurysm and dissection, with high attendant mortality.(24) Heterozygous Arg179 *ACTA2* mutations are associated with a distinctive cerebrovascular phenotype(8) with proximal dilatation of carotid arteries and widespread distal occlusive arteriopathy. Unlike MM, basal collaterals are absent and intracranial arteries are abnormally straight and "broom-like" (figure 1). Clinical features of smooth muscle dysfunction include congenital mydriasis and patent ductus arteriosus (PDA), pulmonary hypertension, bladder and bowel dysfunction, and *livedo reticularis*. It is notable that the vascular phenotype may be influenced by regional differences in arterial structure, namely dilatation of (proximal) elastin-containing segments of the internal carotid artery and stenosis in (distal) elastin-deficient segments.(25) We speculate this may be an example of the local environment interacting with genetic background.

MYH11

Disruptions in the smooth muscle myosin heavy chain 11 (MYH11), also part of the smooth muscle cell contractile apparatus, are associated with thoracic aortic aneurysm or aortic dissection and PDA. The *MYH11* phenotype has been expanded to include MM-like occlusive cerebrovascular disease.(26)

Both dominantly inherited disorders carry attendant genetic implications for family members and aortic screening.

Mutations affecting vascular basement membranes: *COL4A1* and *COL4A2*

COL4A1 and *COL4A2* sit head-to-head on chromosome 13q34, encoding the alpha-1 and alpha-2 chains of type IV collagen, the main component of vascular basement membranes. Autosomal dominant mutations in *COL4A1* result in variable clinical manifestations, including pediatric cerebral small vessel disease. *COL4A2* mutations produce the same phenotype that includes both ischaemic and hemorrhagic stroke. Presentation may be with early-onset hemiparesis, porencephaly and seizures, or with stroke later in childhood or adulthood. Familial heterogeneity is common.(27)

Hemorrhage risk is life-long, and often has an environmental trigger, such as minor trauma.(28, 29) Although there is no specific treatment, diagnosis is useful as patients can be counselled to avoid contact sports and anticoagulation, and mothers of affected fetuses could undergo planned Cesarean section to reduce hemorrhage risk related to birth trauma.

Deficiency of adenosine deaminase 2 (DADA2)

DADA2 is a rare pediatric genetic arteriopathy caused by loss of function recessively inherited mutations in *CECR1* (cat eye syndrome chromosome region, candidate 1), resulting in an autoinflammatory vasculitis. DADA2 leads to abnormal endothelial and leukocyte development and differentiation.(30) A polyarteritis nodosa-like presentation is common. Systemic inflammatory features include livedoid rash, intermittent fevers, hypertension, raised inflammatory markers, and hypogammaglobulinaemia. Neurological features include lacunar and hemorrhagic stroke, peripheral and cranial neuropathies.(30,31) As many mutations in *CECR1* have been described, with wide phenotypic variation, pathogenesis should be confirmed by ADA2 functional enzyme assay.(31) Anti-TNF-alpha therapy is useful as ADA2 functions as an important regulator of immune development.(31)

Environmental associations with childhood AIS and cerebral arteriopathy

While genetic disorders may confer susceptibility to arteriopathy, environmental factors like infection and trauma may be important "second hits" to express the phenotype, or to precipitate AIS in a child with a pre-existing arteriopathy. The VIPS study has provided major insights into the role of infection and inflammation in AIS and arteriopathy. This was a rigorous, NIH-funded international study of 355 prospectively enrolled children with AIS and 354 age-matched controls, including review of clinical and radiographic information, serum sampling, and follow-up for at least one year;(32) findings will be discussed below.

Inflammation in childhood cerebral arteriopathy and AIS

Inflammation is triggered by tissue injury from any cause, such as infection, when inflammatory cells helpfully destroy infected host cells in a transient and localised response. Inflammation becomes pathological when it is disproportionate to the tissue injury, occurring at a distant site, or of prolonged duration.(33) Inflammation contributes to AIS pathogenesis through several mechanisms. Circulating immune mediators can trigger activation of the coagulation system and platelet aggregation, promoting thrombus formation. This could compound other AIS risk factors: for example, inflammation could promote intracardiac clot formation in a child with congenital heart disease. Inflammation can also mediate arterial endothelial injury, disrupting vascular homeostasis, potentially leading to arteriopathy. Infection has been associated with impaired vascular endothelial function in children.(34) In turn, impaired endothelial function may be associated with recurrent AIS in cerebral arteriopathy - circulating indices of endothelial function in a cohort of children with AIS and

cerebral arteriopathy were different between those with a monophasic course compared with recurrent AIS. Children with recurrent AIS had significantly higher markers of endothelial injury, thought to reflect ongoing vessel wall damage,(35) as well as biomarkers of impaired endothelial repair response.(36) Ischaemia itself is a trigger for inflammatory cascades in the brain, resulting in cell death and further inflammation, exacerbating the ischaemic injury.(37)

Laboratory and imaging biomarkers of inflammation in pediatric cerebral arteriopathy

The VIPS study hypothesized that children with arteriopathic AIS would have a distinct pattern of inflammatory biomarkers compared to children with cardioembolic or idiopathic AIS, and that these would predict arteriopathy progression and recurrent AIS. Elevated inflammatory biomarkers in AIS suggest the presence of ongoing inflammation,(38, 39) although could be a result of downstream effects of ischaemia. To minimize the effects of AIS itself, the VIPS study analyses were adjusted for infarct size, seizures and timing of sample collection. The study found that serum levels of three of the four inflammatory biomarkers measured differed by AIS etiology. The cardioembolic group had higher concentrations of high-sensitivity C-reactive protein (hs-CRP) and myeloperoxidase than other groups; the cardioembolic and arteriopathic groups had higher serum amyloid A (SAA) than the idiopathic group. In the arteriopathic group, higher hs-CRP and SAA predicted recurrent AIS.(40) Although speculative, these differences in biomarkers could reflect the varied role of inflammation in AIS, e.g. prothrombotic in children with structural heart disease, or promoting endothelial injury in arteriopathy, as discussed above.

Classification of arteriopathy currently relies on luminal imaging techniques ─ magnetic resonance angiography (MRA), computed tomography angiography (CTA), and digital

subtraction angiography (DSA) ─ which give little information on the disease process in the arterial wall. Vessel wall imaging (VWI) is an emerging technique which directly images the arterial wall using high-resolution MRI. Vessel wall enhancement may represent inflammation(41) and, although requiring validation, VWI may help predict progressive arteriopathy in childhood AIS(42). Further work is needed to fully understand the investigative role of VWI in childhood.

Infection, vaccination and childhood AIS

Although unsurprisingly central nervous system and systemic infection are associated with AIS, there is now clear evidence that trivial viral infection transiently increases risk of childhood AIS. Up to one third of cases of pediatric AIS report infection in preceding weeks.(1, 43, 44) In the VIPS cohort, infection in the week preceding stroke (or interview for controls) conferred a 6-fold increased risk of AIS. Infections were mostly upper respiratory tract, and were common across all stroke subtypes, with a low use of vasoactive cold remedies.(45) A 6-fold increase in AIS risk still results in a low absolute risk, so additional factors are likely to be involved.

Interestingly, the VIPS study found that under-vaccination was an independent risk factor for AIS in an age-adjusted multivariate logistic regression model, odds ratio 8.2.(45) This is despite most infections being identified as upper respiratory tract, which are not vaccinated against. Vaccination could have a broader immune benefit which effects the inflammatory response, or indirectly prevent additional infections.(46)

Further research in the VIPS II study will attempt to better understand the paradox of a common risk factor like childhood infection and a rare outcome like childhood AIS. It will use next generation sequencing for broad, unbiased detection of pathogens, testing the hypothesis that unusual combinations of infections, or unusual strains of infection, explain this exposure paradox. It will also use multiplex bead array testing to measure serum levels of 16 immune mediators, testing the hypothesis that the paradox is explained by an abnormal inflammatory response to infection.(47)

Specific bacterial infections

Severe bacterial infections can be complicated by AIS because of activation of the coagulation cascade, septic emboli, vascular tissue injury and inflammation. Bacterial meningitis can result in a secondary vasculitis as the basal cerebral arteries are exposed to purulent exudate and direct spread of inflammation. Although vaccinations have dramatically reduced the incidence of bacterial meningitis in developed countries, *Salmonella* species and *Streptococcus pneumoniae* were the most common cause of pediatric meningitis complicated by AIS in one large prospective study.(48) Stroke is a common complication of tuberculous meningitis, occurring in one-third of cases, due to a particularly aggressive inflammatory/infectious basal vasculopathy.(49) Other bacterial agents reportedly associated with stroke include *Mycoplasma pneumonia*, *Borrelia burgdorferi*, *Haemophilus influenza*, and *Chlamydia pneumoniae*.(33)

Specific viral infections

Post-*varicella zoster* (VZV) arteriopathy typically affects previously healthy young children, 3-4 months following chickenpox infection (within 12 months to fit diagnostic criteria). VZV infects the trigeminal nerve, and from there gains access to cerebral vessels, directly invading vessel walls causing an inflammatory response, with enhancement on VWI, CSF pleocytosis, and lymphocytic infiltration on biopsy.(37) The radiological pattern is of focal stenosis of the proximal middle cerebral artery and basal ganglia infarction.(50) This is in contrast to VZV vasculitis, largely described in adults, which occurs following VZV reactivation (herpes zoster). VZV vasculitis presents with more diffuse neurological features, has a less consistent angiographic pattern, often with patchy multifocal involvement.(33) Less commonly aneurysms and dissection occur. VZV DNA or, more helpfully, anti-VZV antibodies in CSF aid diagnosis. Anti-inflammatory and anti-viral treatment (corticosteroids and acyclovir) are recommended. In children with the typical clinical and radiographic findings of post-*varicella* arteriopathy, CSF studies are not always performed, and evidence for benefit of treatment of positive findings of VZV DNA or antibodies is lacking. The overlap between these entities remains open (figure 2).

The VIPS study performed herpesvirus serologies in 326 acute AIS cases, including 187 paired acute and convalescent samples. Serological evidence of acute herpesvirus infection doubled the risk of AIS, across all subtypes (even after adjusting for age, race, and socioeconomic status). Almost half of children with paired samples had serological evidence of acute herpesvirus infection. *Herpes simplex* virus type 1 was most common, and most infections were asymptomatic.(51) This association could be partly explained by similar mechanisms to VZV arteriopathy. In addition, a common mechanism across AIS subtypes is theorised: inflammatory endothelial injury promoting cardiac thrombus or cerebral arteriopathy.

Stroke occurring in children with Human Immunodeficiency Virus 1 (HIV1) infection is likely to be multifactorial in origin. Direct HIV1 infection of the arterial wall may cause arteriopathy. AIS may also result from opportunistic infection, drug effects (toxicity, dyslipidaemia, atherosclerosis), prothrombotic factors and inflammatory components.(52)

Other viral infections such as parvovirus, enterovirus and *influenza* A have been reported in the setting of childhood AIS. It is likely that some associated viruses contribute to stroke risk via common mechanisms, activating both proinflammatory and prothrombotic pathways.

Trauma

Significant head trauma (requiring medical attention) in the previous week conferred an almost 40-fold increased odds of AIS compared with matched controls without trauma exposure in a large population-based study. More severe trauma was more strongly linked to AIS.(44) Mechanisms of AIS following major head trauma may be carotid or vertebral artery trauma or dissection, or vasospasm.(53)

Arterial dissection and AIS following minor head trauma may be due to a more complex interplay of factors, such as local inflammation of blood vessels primed by infection or genetic predisposition. While several rare hereditary connective tissue disorders (e.g., Marfan's syndrome, Ehlers-Danlos type IV) confer a particularly high risk of dissection, electron microscopy of skin biopsies in patients with arterial dissection reveal a high proportion with structural abnormalities of collagen fibrils and elastic fibers.(54, 55) Patients with cervical arterial dissection are also more likely to have clinically detectable connective

tissue abnormalities, such as joint laxity or hyperextension.(56) Case-control studies in adults provide evidence of an association between minor acute infection and arterial dissection.(57, 58) Taken together, these studies support the hypothesis that environmental exposures like trauma and infection can act as triggers for arteriopathy (in this case, dissection) in the genetically predisposed.

A distinctive clinical phenotype of basal ganglia infarction following minor injury has been reported in in infants, postulated to result from vasospasm in lenticulostriate perforator arteries.(59) Lenticulostriate artery mineralisation has been described in one cohort,(60) possibly reflecting an underlying vulnerability, but has not been replicated elsewhere.

Treatment

Recurrence rates in childhood AIS with arteriopathy are high, despite antithrombotic therapy. In VIPS, 21% of children with definite arteriopathy had recurrence (highest in MM), compared to 4.5% with idiopathic AIS and 8.1% for cardioembolism.(7) Improved management of arteriopathy, including prevention of recurrence, is a priority in childhood AIS.

As we have illustrated, inflammation appears to be a key mechanism of AIS and recurrence, and, therefore may underlie arteriopathy progression and/or recurrence. Anti-inflammatory therapies are an obvious therapeutic target but there are safety concerns in the context of recent infection. Whilst anecdotally corticosteroids have been used in post-*varicella* arteriopathy and focal cerebral arteriopathy of childhood (FCA), a recent systematic review

of 34 studies concluded that robust evidence of efficacy is lacking.(61) A recent physician survey concluded that a clinical trial of corticosteroids in FCA is a priority (with concomitant use of acyclovir in post-varicella arteriopathy).(62) However, a recent analysis of 84 UK AIS cases did not find a high recurrence rate in FCA.(63) The variable recurrence rate in different cohorts, and in different AIS subtypes, challenges the concept of grouping all AIS cases together for an interventional trial. Rigorous identification of homogenous disease entities and mechanism-specific trials in subgroups would be a more logical approach.

Therapies which inhibit smooth muscle cell proliferation is another potential avenue to explore in pediatric cerebral arteriopathy, particularly in those with genetic smooth muscle dysfunction syndromes, for example imatinib or statins may have a modifying effect.(8)(64)

Improving rates of routine childhood vaccination should be encouraged as a primary stroke prevention method. *Varicella zoster* vaccination is recommended by the WHO in counties who assess a significant public health burden from VZV(65), however it is not part of the UK routine vaccine schedule.

VIPS II will test for a large number of immune mediators to delineate the pathway of inflammation in different AIS subtypes.(47) Understanding the inflammatory response better could lead to more targeted immunotherapy in future. This study also aims to advance understanding of the role of specific pathogens in arteriopathy which could also guide treatment with antibiotics or antiviral therapy.

Conclusion

We have outlined some single gene disorders which are associated with pediatric AIS to illustrate multifactorial aetiology of arteriopathy and the interplay between genetic and environmental factors. The latter are common, for example minor infection or trauma. Inflammation is an important mechanistic final common pathway. A trial of corticosteroids in FCA is planned, but a "one-size-fits-all" approach to pediatric AIS trials should be applied with caution as differing phenotypes have varying disease trajectories, further varying between cohorts. Further defining specific arteriopathy subtypes, and disease-specific targets for trials, should be an immediate priority.

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None

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Table 1: Diagnostic clues in single gene causes of pediatric cerebral arteriopathy

		Genetic mutation **(disease name given below if different)**
History		
Family history	Stroke	AD conditions: *COL4A1, COL4A2, NF1, NOTCH3, SLC2A10, JAG1* AR conditions: *HBB*
	Cardiac disease / TAAD	AD conditions: *ACTA2, MYH11*
	Neurocutaneous	AD conditions: *NF1*
	Consanguinity (or negative family history)	AR conditions: *GUCY1A3, CECR1, HTRA1, SAMHD1, PCNT, ABCC6, HBB*
	Sickle cell trait	*HBB*
	Maternal inheritance	XLR conditions:

		CBS, GLA, ATP7A, BRCC3/MTCP1
Neurological symptoms	Migraine	*COL4A1, COL4A2, NOTCH3*
	Congenital hemiplegia	*COL4A1, COL4A2*
	Developmental delay	*BRCC3/MTCP1, ELN, JAG1, SAMHD1, PCNT* (motor), *ATP7A, CBL, HBB*
	Neuropathies	*CECR1, HBB*
	Seizures	*NF1, ATP7A, SAMHD1, HBB*
	Neurofibromata	*NF1*
	Behavioural / psychiatric	*NOTCH3, HTRA1*
	Encephalopathy, regression	*SAMHD1, ATP7A*
	Acroparaesthesia	*GLA*
Other	Intermittent fever	*CECR1*
	Achalasia	*GUCY1A3*
	Vaso-occlusive crises, anaemia, acute chest syndrome	*HBB*
Examination		
Musculoskeletal	Tall stature	*CBS*
	Short stature	*BRCC3/MTCP1, PCNT, HBB*
	Scoliosis	*NF1*
	Contractures, arthropathy	*SAMHD1, HBB*

	Joint laxity	*ATP7A, SLC2A10*
	Arachnodactyly	*SLC2A10*
	Cramps	*COL4A1, COL4A2*
Skin	Livedo reticularis	*ACTA2, GUCY1A3, CECR1,*
	Raynaud syndrome	*GUCY1A3, SAMHD1*
	Chilblains	*SAMHD1*
	Axillary & inguinal freckling	*NF1*
	Café-au-lait spots	*NF1, PCNT*
	Increased laxity	*ABCC6*
	Angiokeratoderma	*GLA*
	Pallor, jaundice	*HBB*
Hair	Alopecia	*HTRA1*
	Sparse, friable	*ATP7A*
	Premature hair graying	*BRCC3/MTCP1*
Eyes	Cataracts	*COL4A1, COL4A2, BRCC3/MTCP1, GLA*
	Retinal tortuosity	*COL4A1, COL4A2*
	Congenital mydriasis (pupils fixed & dilated)	*ACTA2*
	Optic glioma, lisch nodules	*NF1*
	Peau d'orange angioid streaks	*ABCC6*

	Neovascularisation, Hemorrhage	*ABCC6, HBB*
	Posterior embryotoxon	*JAG1*
	Dislocated lens	*CBS*
Facial dysmorphism	Hypertelorism, long philtrum, mild ptosis	*BRCC3/MTCP1, CBL*
	Small upturned nose, long philtrum, wide mouth, full lips, small chin	*ELN*
	Microcephaly, abnormal teeth	*PCNT*
Abdomen	Splenomegaly	*HBB*
Systemic investigation findings		
Cardiovascular	Arrhythmia	*COL4A1, COL4A2, GLA*
	Supravalvular aortic stenosis	*ELN*
	PDA	*ACTA2, MYH11, JAG1*
	TAAD	*ACTA2, MYH11, CBS*
	Coronary artery disease	*ACTA2, BRCC3, GLA*
	Pulmonary hypertension	*ACTA2*
	Peripheral pulmonary stenosis	*JAG1*
	Dilated cardiomyopathy	*BRCC3/MTCP1*

	LVH, high output heart failure	*HBB*
	Systemic hypertension	*BRCC3/MTCP1, GUCY1A3, CECR1*
	Peripheral artery disease	*ABCC6*
Renal	Cysts	*COL4A1, COL4A2*
	Hematuria, renal failure	*COL4A1, COL4A2, HBB*
	Proteinuria, renal tubular dysfunction	*GLA*
	Bladder dysfunction	*ACTA2*
Hepatic	Cholestasis, bile duct paucity	*JAG1*
Skeletal X rays	Sphenoid dysplasia	*NF1*
	Butterfly vertebrae	*JAG1*
	Spondylosis deformans	*HTRA1*
	Skeletal dysplasia	*PCNT*
Blood investigations	Low platelets	*GUCY1A3*
	Raised HbF on hemoglobin electrophoresis	*HBB*
	Raised inflammatory markers, hypogammaglobulinaemia	*CECR1*
	Raised Creatine kinase	*COL4A1, COL4A2*

Brain imaging		
	Porencephaly	*COL4A1*, *COL4A2*
	Infarction and hemorrhage	*COL4A1*, *COL4A2*, *ATP7A*, *GLA* (PCAIS more common), *CECR1* (lacunar infarcts), *ABCC6* (lacunar infarcts), *HBB*
	White matter signal abnormalities preceding symptom onset	*NOTCH3*, *HTRA1*
	Unidentified bright objects, hamartomas	*NF1*
	Basal ganglia calcification, leukoencephalopathy	*SAMHD1*
	Craniosynostosis, Chiari malformation	*SLC2A10*
Arterial imaging		
	MM, aneurysm or stenosis	*COL4A1*, *COL4A2*, *NF1*, *GUCY1A3*, *JAG1*, *SAMHD1*, *PCNT*, *HBB*
	MM or stenosis	*ELN*
	MM-like with absent basal collaterals & abnormally straight intracranial arteries	*ACTA2*
	MM-like, straight intracranial arteries, profuse basal collaterals	*MYH11*

	MM	*BRCC3/MTCP1*
	MM with posterior circulation involvement	*GUCY1A3*
	Tortuous cerebral arteries	*ATP7A*
	Intra & extra-cranial dissections and aneurysms	*SLC1A10*

AD = autosomal dominant, AR = autosomal recessive, XLR = X linked recessive, PDA = patent ductus arteriosus, TAAD = thoracic aorta aneurysm and dissection, PCAIS = posterior circulation arterial ischaemic stroke, MM = moyamoya

NF1 = Neurofibromatosis type 1, NOTCH3 = Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), SLC2A10 = arterial tortuosity syndrome, JAG1 = Alagille syndrome, HBB = sickle cell disease, CECR1 = adenosine deaminase 2 (ADA2) deficiency, HTRA1 = Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), SAMHD1 = Aicardi-Goutieres syndrome, PCNT = Microcephalic osteodysplastic primordial dwarfism type II, ABCC6= Pseudoxanthoma elasticum, CBS = Homocysteinuria, GLA = Fabry disease, ATP7A = Menke disease, ELN = Williams syndrome

Figure legends

Figure 1: Contrasting appearances of ACTA2 and Moyamoya disease

Catheter cerebral angiograms (frontal projection) with right and left ICA injections. Top row from a patient with R179H mutation in *ACTA2* showing the typical appearances of proximal

ICA ectasia, distal ICA occlusive disease and “twig-like” distal ICA branches. There are no basal “moyamoya” collaterals. In contrast, the bottom row shows images from an otherwise healthy girl with moyamoya disease. There is marked occlusive disease of both terminal ICAs with “moyamoya” collaterals.

Figure 2: Arteriopathy following *Varicella zoster* (VZV) infection

Axial T2-weighted brain MRI (a) and time of flight MRA (b) of the circle of Willis from an 8-year-old girl who presented with transient right-sided weakness. She had had uncomplicated chickenpox 3 months earlier. (a) shows multifocal areas of infarction in the territory of the left middle cerebral artery (MCA); (b) shows a focal area of flow abnormality in the proximal left MCA. She was otherwise well with no systemic markers of inflammation. A diagnosis of focal cerebral arteriopathy was made and she was treated with aspirin (5mg/kg/day). Two weeks later she presented with a more profound episode of right hemiparesis and dysphasia. There was no change on brain MRI; however, the MRA (c) showed a more extensive abnormality in the left MCA, confirmed on catheter angiography that also revealed occlusion of the right A1 segment (d). CSF examination showed no cells but a positive VZV PCR and VZV IgG. She was treated with intravenous acyclovir for 2 weeks then oral acyclovir for 3 months in total. She had no further clinical events; re-imaging was stable and repeat CSF examination unremarkable.