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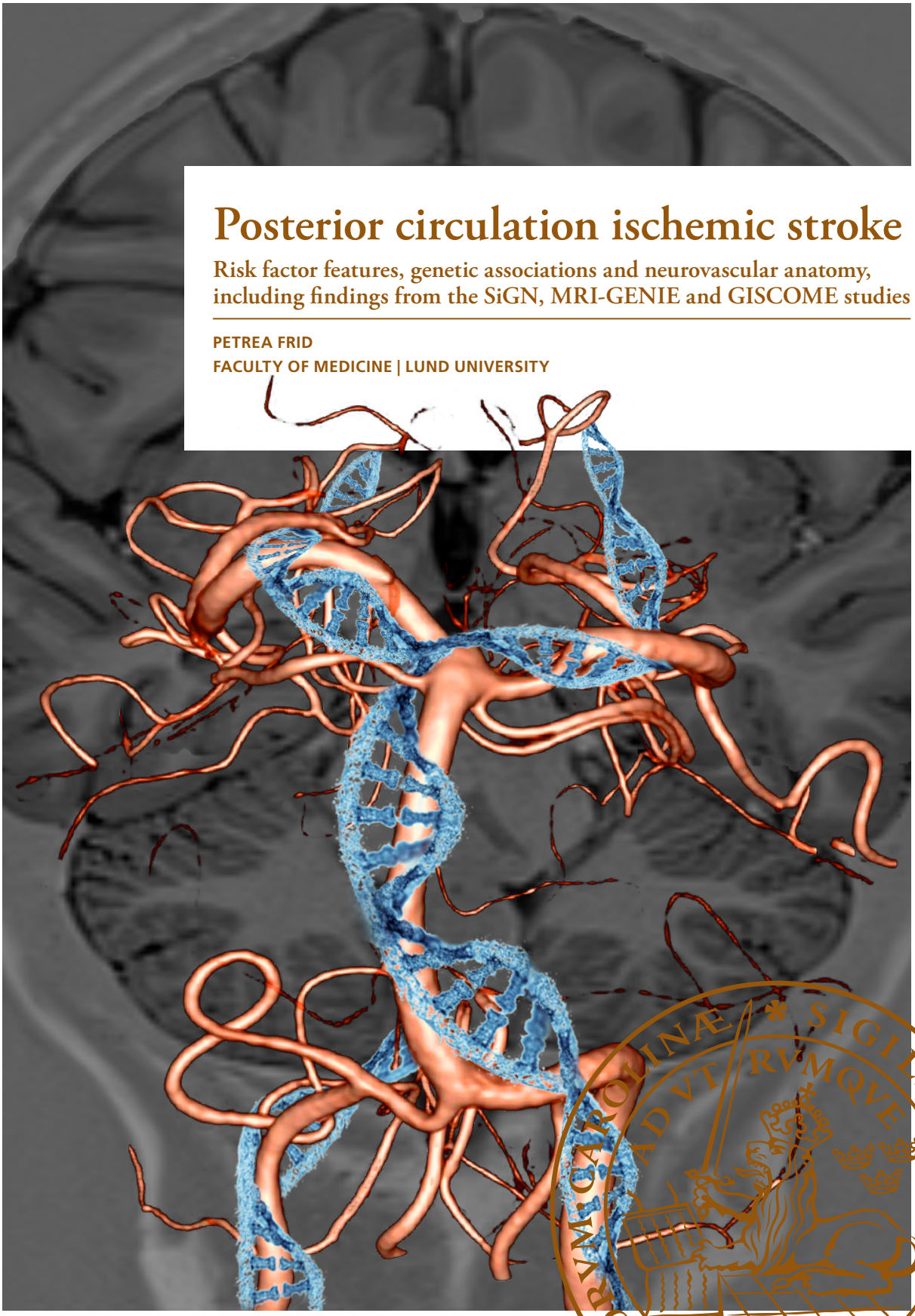
FACULTY OF MEDICINE | LUND UNIVERSITY



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Title and subtitle Posterior circulation ischemic stroke.
Risk factor features, genetic associations and neurovascular anatomy, including findings from the SiGN, MRI-GENIE and GISCOME studies

Abstract

Background: Posterior circulation ischemic stroke (PCiS) is an ischemic stroke type in which the specific influence of known vascular stroke risk factors and pathological mechanisms in relation to anterior circulation ischemic stroke (ACiS) is still unclear. Treatment and secondary prevention options for PCiS are more limited than for ACiS. Investigations into risk factor association and underlying biological mechanisms influencing the risk of PCiS may improve PCiS management.

Aims: We aimed to investigate differences in risk factors and ischemic stroke subtype in an ischemic stroke population diagnosed with MRI-DWI as PCiS or ACiS and to use these enriched image-based stroke phenotypes to evaluate differences in genetic contribution to the risk of PCiS vs. ACiS. Our aim was also to compare the prevalence and laterality of a fetal posterior cerebral artery (FTP) in our ischemic stroke population with a mixed hospital-based population investigated with CTA.

Methods: 3 301 MRI scans in a multicenter ischemic stroke population were evaluated. Patients were phenotyped as PCiS or ACiS based on the localization of ischemic lesions on MRI-DWI. Ischemic stroke subtype, vascular stroke risk factor, and genotype data were available from all centers and used for statistical analyses. For the genetic association study, migraine polygenic risk scores (PRSs) were constructed from migraine GWAS summary statistics. The association of migraine PRSs were compared in PCiS vs. ACiS and separately vs. non-stroke control subjects. FTP prevalence and laterality were evaluated on MRA in the ischemic stroke population and on CTA in a hospital-based population.

Results: PCiS defined as acute ischemic lesions on MRI-DWI occured in 30% of IS patients. The most common etiologies in PCiS were large artery and small vessel disease. Brainstem location of ischemic lesions were strongly associated with small vessel disease while supratentorial lesions were more commonly caused by distal embolism. Diabetes mellitus was independently associated with PCiS vs. ACiS (OR 1.26; 95%CI 1.03–1.55) as was male sex. We found a stronger association with PCiS vs. ACiS for PRSs of two migraine phenotypes. Differential association of migraine PRSs with PCiS vs. ACiS remained when evaluated in PCiS and ACiS separately vs. non-stroke control subjects. There was no difference in the prevalence of FTP between the ischemic stroke and the unselected hospital-based populations. FTP was significantly more common on the right side than the left.

Conclusions: Vascular risk factors differ in prevalence and strength of association between PCiS and ACiS. There may be mechanisms of diabetic injury specifically affecting the vessel wall endothelium of the vertebrobasilar arteries. Such mechanisms remain to be elucidated. The genetic overlap between migraine phenotypes and PCiS indicate shared genetic risk and pathomechanisms in these conditions. A fetal-type posterior cerebral artery is present in up to one third of ischemic stroke patients and unselected hospital-based patients alike, but FTP is not likely to increase the risk of ischemic stroke in the absence of other risk factors. The preferential lateralization to the right of FTP may be associated with other variations in vertebrobasilar anatomy arising during fetal development of the cerebral circulation.

Key words Posterior circulation, ischemic stroke, risk factors, genetic associations, neuroimaging, MRI-DWI

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To my father Bo

Of all the pitfalls in our paths and the tremendous delays
and wanderings off the track
I want to say that they are not what they seem to be.
I want to say that all that seems like fantastic mistakes,
all that seems like error is not error;
and it all has to be done.
That which seems like a false step is the next step.

– Agnes Martin –
American painter

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IV

P. Frid, J. Wassélius, M. Drake, O. Wu, J. Petersson, N.S. Rost, A. Lindgren. *Prevalence and laterality of fetal posterior cerebral artery. A comparison between ischemic stroke patients and an unselected hospital population.* Manuscript.

Abbreviations

List of abbreviations used frequently in this thesis. Abbreviations used infrequently are explained at first mention and are not listed below.

(A)IS	(Acute) ischemic stroke
ACiS	Anterior circulation ischemic stroke
BA	Basilar artery
CCS	Causative classification of stroke
CE	Cardioembolism
CTA	Computed tomography angiography
CoW	Circle of Willis
DNA	Deoxyribonucleic acid
DWI	Diffusion-weighted imaging
FTP	Fetal-type posterior cerebral artery
GWA	Genome-wide association
GWAS	Genome-wide association study
ICA	Internal carotid artery
LAA	Large artery atherosclerosis
LAS	Large artery stroke
M	Migraine
MA	Migraine with aura
MCA	Middle cerebral artery
MO	Migraine without aura
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MRI-GENIE	MRI-Genetics Interface Exploration study
PCA	Posterior cerebral artery
PFO	Patent foramen ovale

PCiS	Posterior circulation ischemic stroke
PRS	Polygenic risk score
SAO	Small artery occlusion
SVO	Small vessel occlusion
SiGN	Stroke Genetics Network
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variant
SVD	Small vessel disease
VA	Vertebral artery

Introduction

Background

Stroke can be broadly categorized into ischemic and hemorrhagic stroke. Ischemic stroke is the cause of 80%–85% of all strokes. Intracerebral and subarachnoidal hemorrhage account for 15% and 5% respectively. Stroke is a global health problem and a major cause of death and disability worldwide.[1]

Ischemic stroke is caused by blockage of blood flow and failure of oxygen delivery to the brain. Ischemic stroke is defined as an episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction, and infarction is defined as permanent neuronal cell death due to ischemia that can be detected pathologically, on imaging or by clinical symptoms persisting >24 hours or until death.[2] The increasing use of magnetic resonance imaging (MRI) in the diagnosis of ischemic stroke has proven the clinical time-related definition of infarction to be arbitrary since evidence of ischemic injury can be detected on MRI in a proportion of patients whose symptoms have completely resolved within 24 hours.

Ischemic stroke is common and has many potential causes and pathophysiological mechanisms. General mechanisms are thrombosis, hypoperfusion and embolism. These have underlying causes that can be categorized as small vessel occlusion, large artery atherosclerosis, cardioembolism, dissection, and other rare or unknown causes. Identifying underlying causes and pathogenic mechanisms is essential in the management and treatment of ischemic stroke.

Pathogenic processes culminating in ischemic stroke are influenced by various risk factors. High blood pressure, smoking, diabetes mellitus, atrial fibrillation, and high cholesterol are risk factors for ischemic stroke that have been well established in epidemiological studies.[3] These risk factors are to some degree modifiable through medical intervention or lifestyle changes.[4] Non-modifiable risk factors are age, sex and race/ethnicity. Genetic factors are increasingly being considered in terms of ischemic stroke risk, and are likely related to both modifiable and non-modifiable factors depending on their biological function.[5]

Ischemic stroke can occur in the anterior or the posterior vascular territory of the brain. In some cases, both territories are affected. Historically, research of posterior circulation ischemic stroke has lagged that of anterior circulation ischemic stroke. The discrepancy was in part due to difficulty in diagnosis,[6] lack of appropriate investigation methods, as well as the idea that ischemic events in the posterior circulation are more benign.[7, 8]

Posterior circulation ischemic stroke (PCiS) share risk factors, pathogenic mechanisms and etiologies with anterior circulation ischemic stroke (ACiS) to a large extent,[9-11] but there are anatomical and physiological differences between the vascular beds that may influence pathogenic processes in ways that are yet not well understood. Difficulty in clinical diagnosis in combination with earlier imaging techniques with low sensitivity for posterior circulation ischemia, and heterogeneous diagnostic criteria between studies[12, 13] have contributed to uncertainty about the influence and potential clinical relevance of such differences.

Advancement in imaging techniques, and the wider use of MRI in diagnosis as well as the accelerated pace of genetic studies including use of large-scale collaborative efforts have opened for new approaches in investigating ischemic stroke subtypes such as PCiS.

This thesis investigates several aspects of the posterior circulation and posterior circulation ischemic stroke by taking advantage of combined imaging, clinical and genotype data collected within the framework of multinational collaborations.

The aim of the introduction is to give an overview of the posterior circulation and PCiS, incorporating known and potential differences in relation to ACiS and to contextualize the methods used in the studies constituting this thesis.

Definitions and anatomy

The cerebral circulation is differentiated into the posterior and the anterior circulation territories, and further into arterial supply areas. The supratentorial brain is supplied by three major arteries and their branches. The middle cerebral artery (MCA) and the anterior cerebral artery (ACA) together supply the anterior brain regions and the posterior cerebral arteries (PCA) supplies the posterior brain regions. The vertebral arteries (VA) and the basilar artery (BA) supply the infratentorial brain encompassing the brainstem and cerebellum.

The posterior circulation

The bilateral extracranial VAs normally originate from the subclavian arteries and travel along the cervical spine, then fuse intracranially to become the BA. The posterior inferior cerebellar artery (PICA) normally branches from the VA before the two VAs fuse to become the BA. The BA courses on the ventral side of the brainstem giving off perforating arteries to the brainstem. The BA trunk gives off the anterior inferior cerebellar (AICA) and the superior cerebellar arteries (SCA) before bifurcating into the PCAs. Perforating arteries arise from AICA, PICA and the SCA. The posterior circulation connects to the anterior circulation via the posterior communicating arteries in the circle of Willis (CoW) at the base of the brain.

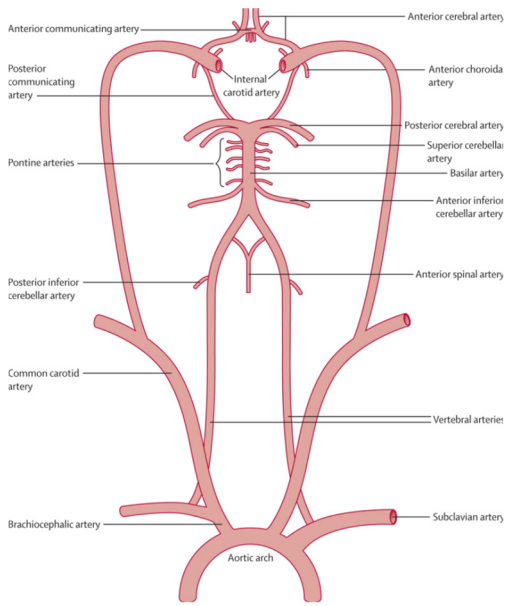

Figure 1. Major arteries of the posterior circulation.
The vertebral and basilar arteries and their branches. The carotid arteries are also pictured. Markus HS, 2013[9]. Reprinted with permission from Elsevier.

Measurements of blood flow volume to the brain has shown that the internal carotid arteries (ICA) of the anterior circulation carry 80% of the brain's blood supply, and the vertebral arteries carry 20%.[14] Anatomical structures supplied by the posterior circulation are shown in Table 1.

Table 1.
Arteries, branches and supply areas of the posterior circulation.

Parent artery and branches		Supply areas
VA	PICA	Inferior posterior surface of cerebellar hemispheres, ipsilateral part of the inferior vermis, dorsolateral part of medulla.[15] Great variation.
BA	AICA	Lateral inferior part of pons, middle cerebellar peduncle, and the flocculus.[15]
	Pontine arteries	Medial and part of lateral pons[16]
	SCA	Superior cerebellar hemisphere, superior vermis, majority of the deep white matter including most of dentate nucleus[15] and part of midbrain.
	PCA	Midbrain, hypothalamus, thalamus. Quadrigeminal plate, geniculate bodies, parahippocampal gyrus, hippocampal formation, parts of the splenium, corpus callosum, inferior side of temporal lobe, and the occipital lobe.[9]

Below is a radiological map correlating brain anatomy on MRI to the vascular territories and arterial supply areas of the brain. The variation between individuals in cerebral vascular anatomy and arterial supply areas is considerable.

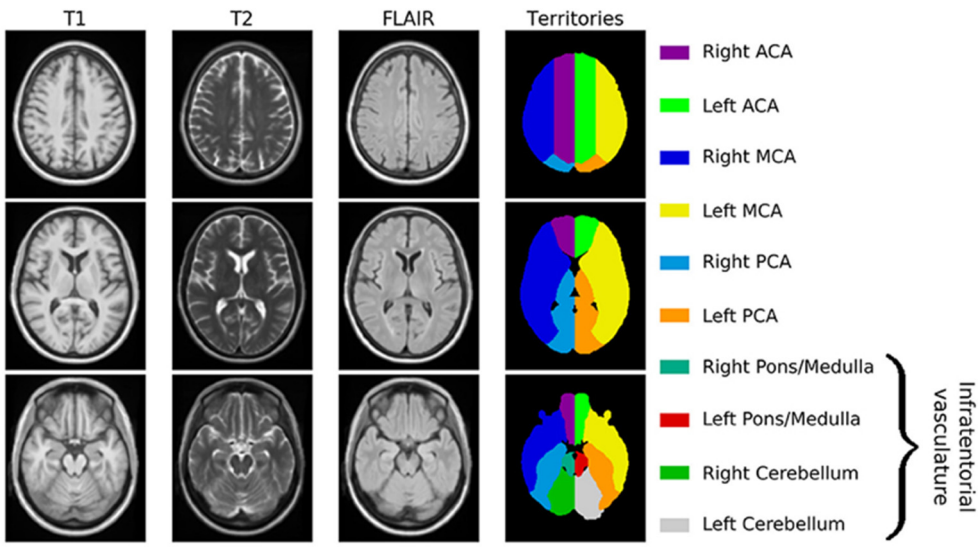

Figure 2.
MRI map of the vascular territories and arterial supply areas of the brain. With permission: www.resilientbrain.org

Posterior circulation ischemic stroke

When a permanent focal ischemic injury occurs in brain tissue supplied by the VA, BA or posterior cerebral arteries (PCA) and their branches and penetrating arteries because of thrombosis, embolism or hypoperfusion, the event is a posterior circulation ischemic stroke (PCiS). In some cases, the supratentorial posterior brain is supplied by the anterior circulation via the ipsilateral ICA and PCiS may therefore sometimes be caused by pathology in the anterior circulation.

Variations in vessel anatomy

Fetal development

The ICAs of the anterior circulation appear on day 24 of embryological development. Four days later the ICA branches into an anterior and posterior portion. The anterior portion gives off the MCA, ACA and anterior choroidal (AChA) arteries, while the posterior portion give rise to the posterior choroidal (PChA) and the PCAs.[17] Up to this point the hindbrain is supplied by carotid-basilar anastomoses. The growth of the occipital lobe and brainstem stimulate the development of an independent posterior circulation with regression of the carotid-basilar anastomoses and budding of the vertebral arteries from the distal end of the basilar artery. As the fetal PCA regresses, the connection between the anterior and posterior circulation remains as the posterior communicating arteries (Pcom) which are then incorporated into the CoW. The adult PCA develops when fetal proximal PCA branches fuse to form the distal BA and the two arteries connect.

Vessel variants

Individual variations in the anatomy of the cerebral arteries are common. Variation in vessel anatomy is more often observed in the posterior than in the anterior circulation.[18] The brain's primary arterial collateral network in the CoW is complete in only a minority of individuals.[19-22] In a meta-analysis of 33 studies the prevalence of variation of the CoW in healthy individuals was 68.22% ± 14.32% (SD).[22]

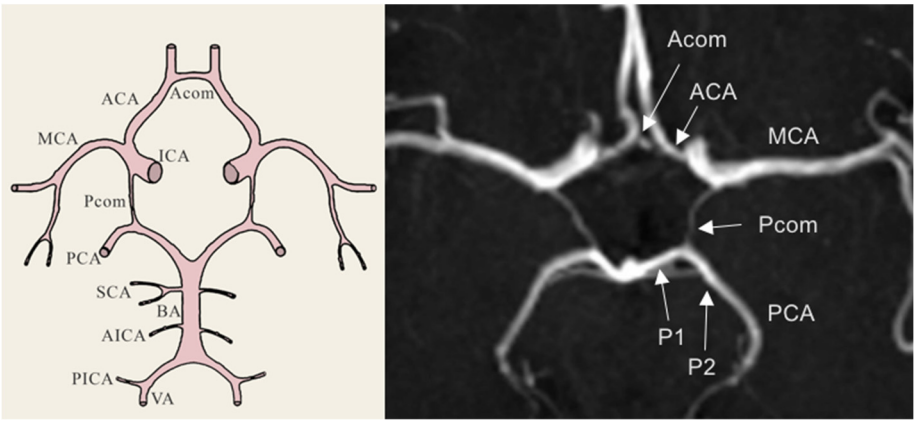

Figure 3.
Complete circle of Willis illiustrated (left) and on MRA imaging (right). The complete configuration is found in a minority of individuals. Acom=Anterior communicating artery; ACA=Antererior cerebral artery; Pcom=Posterior communicating artery; PCA=Posterior cerebral artery; P1, P2 = segments of the PCA.

A frequently found CoW variant is a fetal posterior cerebral artery (FTP) in which the PCA is a branch from the ICA instead of the BA. There is either a small hypoplastic P1 segment (partial FTP) or the P1 segment is absent, in which case there is no connection between the anterior and the posterior circulation (full or complete FTP) (Fig. 4).

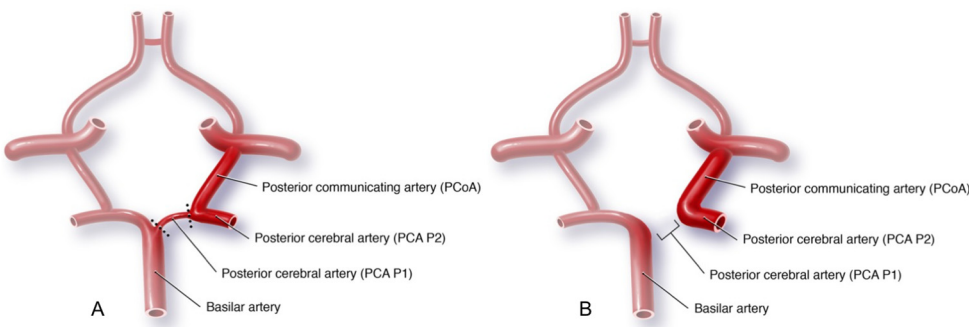

Figure 4.
A. FTP with a hypoplastic P1 segment (partial FTP). **B**. FTP with absent P1 segment (complete FTP). S. Capone, 2019.[23] Reproduced under the Creative Commons Attribution License.

The FTP variant represents a persisting normal embryonic artery[17] and is a remnant of early vascular anatomy. FTP often exists in combination with variants in the anterior part of CoW. Its presence has also been associated with vertebral artery hypoplasia.[24] The trigeminal, hypoglossal, otic and proatlantal arteries are other examples of embryonic vertebrobasilar-carotid anastomoses that can persist beyond birth (figures 5 and 6).

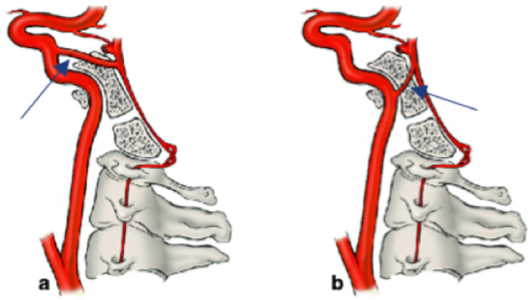

Figure 5.
Persistent embryologic connections between the carotid and vertebrobasilar system. Shown here (arrows) are (a) persistent trigeminal artery, and (b) persistent otic artery. From Harrigan M.R., Deveikis J.P. (2013) Essential Neurovascular Anatomy. Humana Press, Totowa, NJ. Reproduced with permission from Springer.

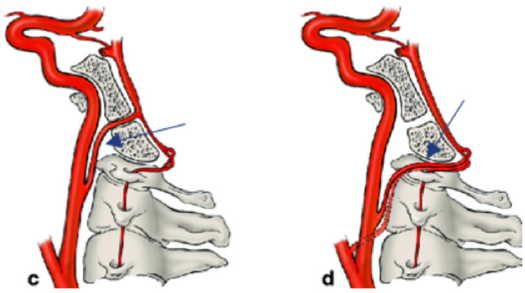

Figure 6.
Persistent embryologic connection between the carotid and vertebrobasilar system. Shown here (arrows) are (c) persistent hypoglossal, and (d) persistent proatlantal artery. From Harrigan M.R., Deveikis J.P. (2013) Essential Neurovascular Anatomy. Humana Press, Totowa, NJ. Reproduced with permission from Springer.

Prevalence data on FTP vary depending on method of detection and definition (partial or complete) used. Recent studies using computed tomography angiography (CTA) or magnetic resonance angiography (MRA) imaging have found a prevalence of 16%–32% in healthy and ischemic stroke populations. Table 2 summarizes prevalence data from recent studies with CTA/MRA. Only a few studies report laterality and this is rarely specifically addressed.

Table 2.
Prevalence of FTP in recent studies using CTA or MRA

Study	Year	Population	Method	Complete	Partial	Complete or Partial
Gaigalaite et al.	2019	Healthy	MRA	—	—	16%
Shaban et al.	2013	Ischemic stroke	CTA/MRA	9.5%	15.1%	—
Arjal et al.	2014	Mixed hospital	CTA	16.8%	10%	26.7%
Coulier B.	2021	Mixed hospital	CTA	9.8%	not reported	—
Krabbe-Hartkamp et al.	1998	Healthy	MRA	—	—	32%

In cases of complete FTP the large artery connection between the carotid and vertebrobasilar systems on the affected side is completely absent and the brain's secondary collateral network of leptomeningeal vessels cannot develop.[25, 26] Secondary collateral vessels are recruited when the primary pathways fail, or under conditions of hemodynamic and metabolic stress of longer duration.[27] The influence of complete FTP on stroke evolution in the acute phase has not been systematically investigated and an increased risk of ischemic stroke in the absence of other specific risk factors has not been established.

Posterior vs. anterior circulation vessel physiology

As described above, the posterior circulation develops after the anterior circulation and is annexed to the supratentorial brain at a later stage during fetal development. The vertebral arteries are developed from mesoderm, while the carotid arterial system develops from the neural crest.[28]

Innervation

Sympathetic and parasympathetic innervation and transmitter release regulating cerebral vascular tone, vasoconstriction and vasodilatation have been described extensively for the anterior circulation.[29-31] There is less knowledge about the sympathetic and parasympathetic innervation and function in posterior circulation arteries. Arteries in the anterior circulation are more densely innervated with sympathetic nerve endings than arteries in the posterior circulation.[32] The perivascular innervation which is a mediator in vascular tone and regulation of cerebral blood flow also differs between the anterior and posterior circulation.[29]

The endothelium

The intracranial arteries, arterioles and capillaries have a single layer of endothelial cells constituting the innermost lining of the vessel lumen, constantly exposed to arterial blood flow. This single layer of cells in the cerebral arteries together with tight junctions protect and maintain the blood-brain-barrier. Endothelial cells partake in the regulation of vascular tone and cerebral blood flow, and part of their normal function also includes inhibition of platelet activation preventing thrombus formation and inflammation.[33] Known and common risk factors for ischemic stroke such as hyperlipidemia, diabetes mellitus and hypertension all cause endothelial injury through complex molecular pathways. Endothelial vulnerability to injury by these mechanisms differ depending on the location throughout the vascular system.[34] Endothelial cells are also transmitters of vasoactive substances and respond to input from perivascular nerve endings to regulate cerebral blood flow.[29]

Nitric oxide (NO) is a pivotal substance with potent vasodilatory effects influencing cerebral hemodynamic regulation.[35] It is synthesized by endothelial cells from L-arginine via endothelial nitric oxide synthase (eNOS). Because of the central role of the endothelial cell in vasodilation and release of vasodilating substances, endothelial dysfunction has been investigated as a potential pathogenic mechanism in conditions such as migraine,[36] posterior reversible encephalopathy[37] and is central in the molecular dysfunction underlying MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes).[38] A few studies have indicated cerebral endothelial dysfunction limited to the posterior circulation in migraine patients[39] and of lower cerebrovascular reactivity to L-arginine in migraine patients compared to control subjects.[40] Dysfunction of the cerebrovascular endothelium may represent a common pathogenic factor in conditions with a predilection for changes in the posterior circulation territory.

General epidemiological and clinical aspects

The true incidence of PCiS in the general population is unclear because few stroke incidence studies specify vascular territory. The NEMESIS study[41] in which patients were classified according to the Oxfordshire Community Stroke Project[42] (OCSP) system found an incidence of 17/100 000 per year. The proportion of PCiS in hospital-based cohorts is usually 20–30% depending on study design, diagnostic and classification criteria and imaging used.[43-46]

Gold standard epidemiological studies on which etiology and risk factor prevalence for ischemic stroke are based often do not specifically investigate PCiS. It is therefore not

clear if the relative contributions of conventional vascular risk factors such as hypertension and diabetes mellitus differ between PCiS and ACiS. Results from studies based on hospital cohorts have heterogeneous diagnostic criteria for PCiS and are difficult to compare. Male sex seem to be more common in PCiS[7, 45] and diabetes mellitus has shown stronger association with PCiS than ACiS in several studies.[7, 13, 45]

Because of the functionally diverse brain structures that are affected in PCiS, the clinical picture is varied and encompass symptoms from mild episodes of vertigo to quadriplegia and coma. There is also a substantial overlap in symptoms between anterior and posterior stroke.[7] The most feared manifestation of PCiS is basilar artery occlusion for which the mortality rate is as high as 80% and functional outcome poor in a substantial portion of survivors even when endovascular treatment is given.[47]

There are other inequities between PCiS and ACiS patients in the clinical setting. PCiS patients risk missed diagnosis[48] and delayed treatment compared to ACiS patients,[49] due to difficulty in recognizing PCiS signs and symptoms. In addition, both CT and MRI imaging detects PCIS with less sensitivity than ACiS,[50, 51] potentially leading to missed diagnosis and lost opportunities for secondary prevention. Recurrence rate is high in vertebrobasilar stroke and transitory ischemic attack (TIA) of atherosclerotic etiology[52, 53] but options for secondary prevention treatment for patients with symptomatic atherosclerosis are fewer than for ACiS and are limited to best medical therapy including antiplatelet drugs. Carotid endarterectomy is a proven, highly effective intervention for preventing recurrent stroke in patients with symptomatic high-grade stenosis of the internal carotid artery (ICA).[54, 55] No corresponding treatment exists for patients with atherosclerotic disease of the VA or BA. Stenting and angioplasty of the VA have not been proven beneficial as secondary prevention measures in clinical trials so far.[56] In addition, PCiS patients have historically often been excluded, underrepresented, or not specifically investigated in randomized clinical trials on acute stroke treatment.

PCiS etiologies and lesion location

Common etiologies in PCiS are the same as in ACiS though their relative proportion differ.[45, 46] They are cardiac embolism, penetrating small artery disease, atherosclerosis, and arterial dissection.[11, 44, 45, 57, 58] Cardiac embolism is proportionally a more common cause of anterior than posterior stroke.[10, 59] Large vessel atherosclerosis is a common PCiS etiology[43, 58, 60] and can cause stroke by embolism following e.g. plaque rupture or by hemodynamic insufficiency due to high-grade stenosis. Common sites of atherosclerotic lesions in PCiS are the extracranial vertebral arteries near the vertebral

artery ostia at its origin from the subclavian arteries, and in the proximal segment running from the origin of the artery to the point of entry into the transverse foramen of the vertebral column.[8, 9, 61] Intracranial atherosclerosis occurs in the basilar artery,[61-63] the intracranial segments of the vertebral arteries[61, 64] and occasionally in the posterior cerebral artery.[61] Stroke as a consequence of small artery disease is relatively more common in PCiS than in ACiS.[43, 45] Compared to the anterior circulation the parenchyma, i.e. brainstem and thalamus, supplied by penetrating arteries make up a larger proportion of the posterior circulation which could partly explain this difference.[59] Cervical artery dissection can cause stroke due to formation of thrombi at the dissection site with secondary embolism to the brain. In young patients, vertebral artery dissection is a common cause of stroke in the posterior circulation.[65, 66] Vertebral artery and carotid artery dissection may have different risk factor profiles and genetic underpinnings.[67]

Unusual and specific etiologies and mechanisms

There are many unusual etiologies of ischemic stroke including haematological disorders, states of hypercoagulability, venous thrombosis, inflammatory vascular disorders, migraine and rare genetic disorders.[68] In PCiS, there are unusual causes such as subclavian steal syndrome[69] and dolichoectasia[70] that cause stroke specifically in the posterior circulation due to vessel pathology near or in the vertebrobasilar system. Other conditions in which a predilection for PCiS have been noted are Fabry's disease, an X-linked lysosomal storage disease[71, 72] and migraine.[73]

Etiology related to lesion location

The location and distribution pattern of ischemic lesions can be related to etiology and stroke mechanism.[59] MRI including MRI-DWI delineates features of AIS with high accuracy and is useful in guiding diagnostic workup and determining stroke etiology.[74-76] Several registries of PCiS patients have reported data on lesion location in relation to etiology and mechanisms.[44, 57, 59] Though characterization of lesion location (vascular supply or anatomy) differs slightly between them, findings are consistent. The cerebellum and PCA territory were the most commonly involved in PCiS and were attributed to cardiac and atherosclerotic sources of embolism, whereas smaller brainstem lesions were either attributed to atherosclerosis or penetrating artery disease.

Classification of ischemic stroke

Determining the underlying cause of an ischemic stroke event is essential for clinical decisions on further work-up, treatment and secondary prevention therapy, but also for categorizing patients into more homogeneous groups in clinical trials, epidemiological studies or for defining stroke phenotypes in genetic research. Several systems for etiological classification of ischemic stroke exist. The most commonly used system is the Trial of ORG 10172 in Acute Stroke Treatment (TOAST),[77] originally developed to classify a large patient cohort in a clinical trial determining treatment effect in AIS. Despite its specific purpose it is widely used outside of the clinical trial setting. The TOAST system classifies ischemic stroke patients into five main categories (Table 3)

Table 3.
TOAST classification of subtypes of acute ischemic stroke.[1]

Large artery atherosclerosis (embolus/thrombosis)*
Cardioembolism (high-risk/medium-risk)*
Small-vessel occlusion (lacune)*
Stroke of other determined etiology*
Stroke of undeterimined etiology
a. Two or more causes identified
b. Negative evaluation
c. Incomplete evaluation

*Possible or probable depending on ancillary studies.

1. Reproduced from Adams HP Jr, Bendixen BH, Kappelle LJ, et al. *Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment.* Stroke. 1993 Jan;24(1):35-41

TOAST was developed further into the Causative Classification of Stroke (CCS) system to address issues of competing etiologies and inter-rater agreement and to improve the usefulness of stroke classification in multicenter settings.[78] CCS is an evidence-based, web-based algorithm taking into account clinical evaluation, results of brain and cerebral vessel imaging, echocardiography, heart rhythm monitoring, and evaluation for uncommon causes of stroke.[79] In contrast to TOAST it assigns a most likely etiology even in cases with multiple potential causes.[79] Main differences between the two classification systems TOAST and CCS are shown in Table 4.

Table 4.
Main differences between CCS and TOAST ischemic stroke subtype classifications[1]

	TOAST	CCS
Year of publication	1993	2005
Diagnosis of LAA	Requires imaging of a limited portion of the extracranial circulation	Result influenced by imaging of intracranial vessels (if performed)
Diagnosis of SAO	No imaging confirmation required	Imaging confirmation required
Size limit for lacunar infarct	15 mm	20 mm
Imaging of the parent artery in lacunar infarcts required	No	Yes
Threshold to separate high- and low-risk cardiac sources	No	2% absolute primary risk threshold
Criteria to identify the most likely etiology in the presence of multiple etiologies	No	Yes
Criteria to identify a known subtype in patients with incomplete evaluation	No	Yes

1. Adapted from McArdle PF et al. *NINDS SiGN Study. Agreement between TOAST and CCS ischemic stroke classification: the NINDS SiGN study*. Neurology. 2014 Oct 28;83(18):1653-60.

The ischemic stroke population in this thesis is derived from 12 centers within the (Stroke Genetics Network) SiGN study.[80] Genetic association studies rely on large samples and consistent phenotyping across many collaborating sites. To maximize consistency in ischemic stroke classification across centers. SiGN adjudicators re-phenotyped all patients in the SiGN study according to CCS.[80] The overall agreement within SiGN between existing TOAST classifications and CCS was moderate, with the lowest agreement for small-artery occlusion and the highest for larger artery atherosclerosis.[81] All patients included in this thesis have available CCS data and comparisons of subtype classification between PCiS and ACiS are based on this system. There is a limited number of studies comparing risk factors and etiological subtypes between PCiS and ACiS based on modern classification systems in patients derived from the same cohort. Several have used TOAST,[45, 46, 58, 65] and a few[12, 13] have used the Oxfordshire Community Stroke Project[42] (OSCP) classification, which is based on clinical criteria. We are not aware of previous studies on PCiS based on stroke classification according to CCS.

MRI-DWI imaging of ischemic stroke and PCiS

This ischemic stroke population studied in this thesis was investigated with MRI diffusion-weighted imaging (DWI) and the diagnosis of PCiS or ACiS is based on DWI lesion location. In contrast to clinical diagnosis, evidence of DWI lesions consistent with PCiS has high diagnostic reliability and can be analysed in relation to clinical characteristics, risk factors and explored as a phenotype in genetic studies.

Magnetic resonance imaging with DWI

Diffusion-weighted MRI is an imaging technique based on the detection of movement of water molecules in (brain) tissue. In ischemic tissue, restricted diffusion of water protons due to cytotoxic edema gives rise to low signal on the apparent diffusion coefficient (ADC) map and hyperintense areas on diffusion-weighted images (DWI).[82] In experimental animals these changes have been detected within a few minutes to one hour after arterial occlusion.[83, 84]

Magnetic resonance with DWI in conjunction with ADC maps identify ischemic injury in acute stroke patients with high sensitivity and specificity[85-88] and is considered gold standard in clinical ischemic stroke diagnosis. DWI lesion size has also been shown to be predictive of final infarct size in anterior circulation stroke.[88, 89] Guidelines issued in 2010 by the American Academy of Neurology established MRI-DWI as the preferred imaging technique in acute ischemic stroke within 12 hours of symptom onset and as the most precise method of establishing ischemic stroke diagnosis.[50]

Despite its high accuracy in the early time window, the evolution and appearance of DWI lesions on MRI in ischemic stroke is variable. Whether the DWI lesion reliably corresponds to permanently infarcted tissue is a matter of debate. Some studies have shown infrequent reversal of DWI hyperintensity after reperfusion therapy on repeated DWI performed up to 1 week post stroke[90, 91] or fluid-attenuated inversion recovery (FLAIR)/T2 weighted sequences up to 90 days post stroke.[92, 93] A recent systematic review concluded that partial reversal of DWI is seen in a quarter of patients and is associated with reperfusion/recanalization therapy, but total reversal is rare.[94]

A persisting increased DWI signal can be visible for weeks after stroke onset.[95] High DWI signal in the acute phase is a result of low ADC values and T2 shine through.[96] ADC values begin to normalize after 7–10 days and finally becomes increased at which stage the DWI signal decreases. A high DWI signal beyond ADC normalization is due to T2 shine through.[97] The anatomical distribution pattern of acute DWI lesions can also be of clinical significance as it may aid in determining stroke etiology and thus

guide patient workup and secondary prevention measures. Stroke etiology according to TOAST criteria has shown a strong relationship with DWI lesion topography[98, 99] and the presence and DWI distribution patterns have also been identified as predictors of early stroke recurrence.[100]

DWI in posterior circulation ischemic stroke

Computed tomography has low sensitivity for ischemic changes within 3–6 hours of stroke onset in all vascular territories.[101] In PCiS, artefacts from the bony structures in the posterior fossa reduces sensitivity even further.[51] MRI-DWI has higher sensitivity, but negative DWI on acute imaging in patients with clinical stroke occurs in up to a third of patients.[102] A high proportion of false negative DWI in PCiS has been reported,[103, 104] in particular in brainstem locations.[105, 106] PCiS is strongly associated with negative DWI (OR 5.1, 95% CI 2.3–11.6, p<0.001) in the acute stage compared to ACiS.[107] MRI-DWI is still the most sensitive imaging method for identifying small and brainstem ischemic lesions that are initially missed on CT.

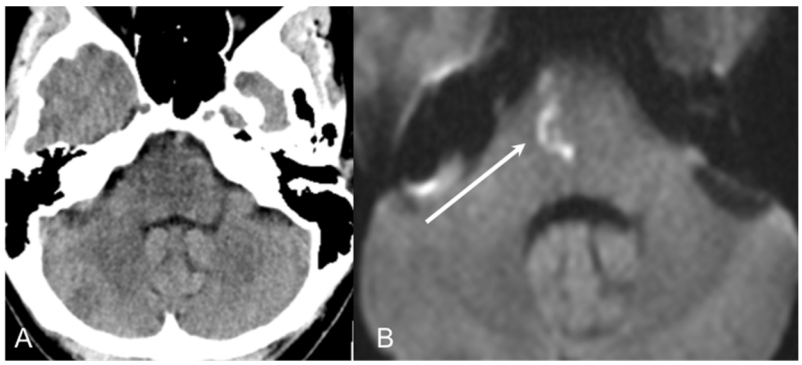

Figure 7.
(A) CT and (B) MRI-DWI of acute ischemic stroke affecting the brainstem. The lesion is not detected on CT but is clearly visualized on MRI-DWI.

MRI-DWI images of acute ischemic stroke

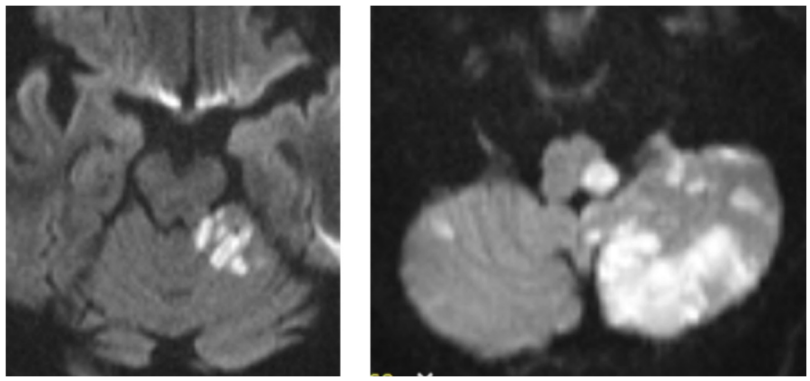

Figure 8.
MRI-DWI showing hyperintense areas corresponding to (A) cerebellar infarction, and (B) ischemic lesions involving the left cerebellum and left dorsal brainstem.

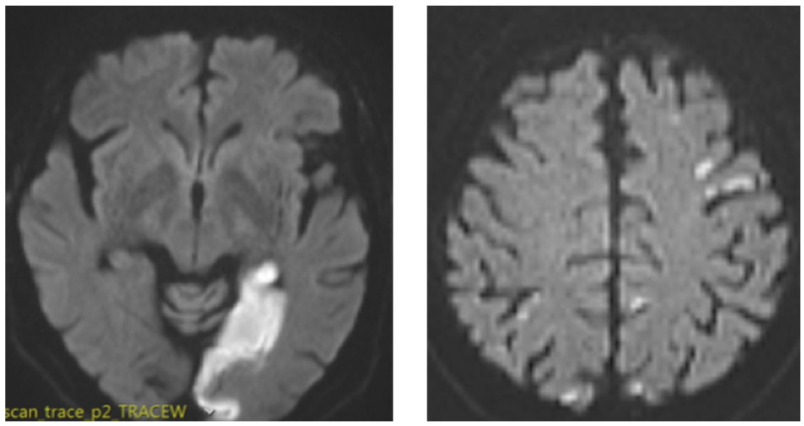

Figure 9.
MRI-DWI showing (A) infarction in the left PCA territory, and (B) multiple and bilateral cortical lesions to both the anterior and posterior vascular territories.

Genome-wide association (GWA) studies

Genome-wide association (GWA) studies can be used to investigate the genome of a large number of individuals for changes or variations in the smallest unit of human DNA—the nucleotide base—that may be associated with the risk of common and complex diseases such as ischemic stroke, migraine and cancer. The GWA method is an agnostic approach in which no a priori assumptions concerning causative biological

mechanisms or molecular pathways need to be made. Other types of genetic association studies often rely on hypotheses of involved molecular pathways and candidate genes.[108]

The human DNA contains approximately 6 billion nucleotide bases arranged into nucleotide base-pairs.[109] When a variation in a single nucleotide base-pair occurs frequently (>1%) within a population it is referred to as a single nucleotide polymorphism (SNP).[110] Such polymorphisms are widely and randomly distributed across the human genome and occur in coding regions, regions regulating gene expression, or in regions between genes.[111] The effect on disease risk conferred by most of these common variant is very small and identified variants only explain a small portion of the heritability of complex diseases.[112, 113] Rare genetic variants with high penetrance that cause monogenic disease are generally not identified in GWA studies.

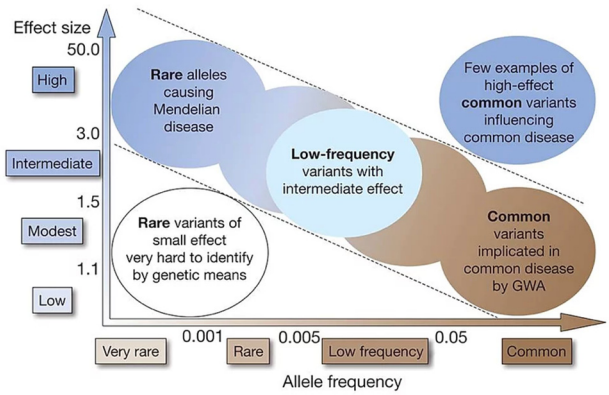

Figure 10.
The figure illustrates the probability of identifying genetic variants by risk allele frequency and strength of genetic effect. From Manolio, 2009.[112] With permission from Springer Nature.

GWA studies of complex diseases are often case-control studies in which the genome of individuals with a given trait or phenotype are compared with that of individuals without the trait or phenotype to identify associations between common genetic variants and the phenotype or disease.[114] A strategy for increasing the sample size and power of GWA studies is to combine the results from several other GWA studies on the same trait and conduct a GWAS meta-analysis.

Ischemic stroke GWA studies

Ischemic stroke is a common, complex and polygenic disease. Common risk factors such as hypertension, atrial fibrillation and smoking are known to increase stroke risk,[115] and a proportion of the risk can be attributed to genetic factors.[116] GWA studies

have identified >35 genomic risk loci associated with ischemic stroke and ischemic stroke subtypes,[117-119] and many more risk loci have been identified for stroke risk factors.[120-122] A challenge in GWA studies of ischemic stroke has been its heterogeneous etiology involving multiple mechanisms such as small and large vessel disease and cardioembolism.[80] To date there has been no GWA studies specifically exploring the association of common genetic variants with PCiS.

Migraine GWA studies

Migraine is also a complex disease caused by multiple environmental and genetic factors. Epidemiological studies indicate that migraine with aura (MA) is more heritable than migraine without aura (MO).[123, 124] GWA studies of migraine phenotypes have consistently found stronger associations with genetic loci for migraine without aura.[125] One proposed explanation for this is that migraine with aura may be more associated with rare risk alleles with higher penetrance.[126] The largest GWAS of migraine to date identified 38 migraine associated loci pointing to genes regulating vascular, neuronal and pain pathways among others.[127]

Neuroimaging in genetic association studies

With advancement in imaging techniques neuroimaging features have increasingly been used to investigate the genetic architecture of conditions such as Alzheimer's disease, Parkinson´s disease and schizophrenia.[128] Evidence of these heritable conditions can be visualized with different MRI techniques and are referred to as endophenotypes or intermediate phenotypes. In cerebrovascular disease, such specific neuroimaging features have been successfully used to explore the genetic architecture of white matter hyperintensities and small vessel disease[129, 130] Ischemic stroke GWA studies have mostly explored common variants associated with specific ischemic stroke etiologies, or stroke phenotypes, such as large artery disease or cardioembolism, which are defined using a combination of clinical and imaging data. An example of how detailed image phenotyping can help delineating stroke subtypes for the purpose of exploring genetic contribution to ischemic stroke risk is a study in which lacunar stroke lesions were categorized according to if MRI showed multiple or isolated lacunar lesions. The study showed that the heritability for lacunar stroke was greater than what had been shown in earlier GWA studies.[131]

Genetic and polygenic risk scores (GRSs and PRSs)

GWA studies have made it clear that complex diseases are often polygenic and influenced by hundreds or even thousands of genetic variants. Individual variants have a small effect on the genetic risk for disease[132] and variants identified in GWA studies as being significantly associated with a trait may also correspond to only a small portion of the number of variants that are truly associated with the disease, which limits their use in clinical risk prediction.[133] To increase the predictive power of GWAS data, methods for evaluating the effects of many SNPs simultaneously by combining them into genetic risk scores (GRS) have been developed.[134] One such score is the polygenic risk score (PRS). PRSs are calculated by summarizing the number of risk alleles in an individual, weighting the sum by the effect size in the original GWAS.

In essence, PRSs reflect an individual's genetic load of a selection of common variants and can be used for predicting risk of disease or for investigating shared etiology between different phenotypes or related diseases.[133, 135]

Risk prediction

Genetic scores applied as clinical tool for risk prediction is still limited. However, there are a few common complex diseases in which PRS prediction have shown real-world clinical utility, In coronary artery disease, PRSs predict an individual's added risk for a coronary event and can help clinicians stratify patients into low to high risk categories for decision making on primary preventive statin therapy.[136, 137]

Similar scores to predict the genetic risk of ischemic stroke have until recently been more difficult to develop due to the heterogeneity of stroke subtypes and a lack of genetic data for large well-defined ischemic stroke populations, but several recent studies have been conducted. In one such study,[138] stroke GRSs were constructed from GWAS results in the MEGASTROKE[117] study to investigate their association with incident stroke and to evaluate if a healthy lifestyle (e.g. exercise, healthy diet and no smoking) would mitigate the genetic risk. GRSs and lifestyle factors were each independently associated with the risk of incident stroke. Results indicated that regardless of lifestyle profile, individuals with the highest genetic risk had a 35% increase in risk of incident stroke compared with those with low genetic risk, but also that an unhealthy lifestyle compared to a healthy one conferred an even greater risk increase (65%) independently of the genetic risk level. For all levels of genetic risk, observance of a healthy lifestyle lowered the risk of stroke. This knowledge is useful for communicating to a population that a healthy lifestyle is beneficial regardless of individual genetic risk.

Another advancement in the construction of genetic risk scores is the method of combining several GRSs into a metaGRS, essentially a composite score representing the additive risk conferred by common variants associated with a number of known risk factors. In one study,[139] such a metaGRS composed of 19 distinct GRSs for ischemic stroke subtypes, coronary artery disease, lipid levels, diabetes mellitus, body mass index and blood pressure was evaluated to test its predictive power for ischemic stroke risk. This metaGRS increased the genomic predictive power compared to individual risk factor GRSs and was estimated to detect even the few individuals (0.25%) with a threefold increase in stroke risk, a risk level equivalent to that of some monogenic cardiovascular diseases. Importantly, the metaGRS was shown to have stronger predictive power than other well-known risk factors, and accounted for the proportion of risk not explained by these risk factors. The IS incidence was increased 1.7%–2.8% in individuals whose risk factor profile was within guidelines but whose metaGRS risk level was in the highest percentile. This type of genetic association studies on ischemic stroke risk have the potential to guide early prevention measures, as well as provide risk stratification for targeted risk factor management in individual patients.

Investigating shared etiology and pleiotropy

Pleiotropy in this context indicates that a genetic variant associates with two or several phenotypes or traits, and implies that the traits share a genetic background or are correlated genetically.[140] Early, successful pleiotropy studies using PRSs to explore shared etiology are found in neuropsychiatric research.[141, 142] An example of PRSs utility in identifying shared etiology in ischemic stroke research is a recent genetic association study of ischemic stroke and migraine in which genetic overlap between migraine phenotypes and ischemic stroke and subtypes were found.[126] The study did not investigate if the genetic overlap with migraine was different for PCiS than for ACiS.

Migraine as a candidate phenotype

The migraine–ischemic stroke association is complex. The biological mechanisms linking migraine and ischemic stroke are most likely multifactorial and bidirectional. We conceptualized PCiS as a distinct stroke subtype to investigate shared genetic contribution in migraine and PCiS and if this contribution differed between PCiS and ACiS. The rationale is based on clinical, genetic and neuroimaging data and is discussed below, together with other findings relating to ischemic stroke and migraine.

Migraine and ischemic stroke

Migraine headache is a common neurological disorder, with a global prevalence of 14%.[143] It has two to three times higher prevalence in women than in men[144] and is most common in midlife.[145] Migraine is characterized by recurring headache episodes with or without aura preceding the attack. Epidemiological data from observational and prospective cohort studies show that migraine, particularly migraine with aura is associated with an elevated risk of ischemic stroke.[146-149] Specific risk factors for ischemic stroke in migraine are young age,[150] female sex,[147] oral contraceptive use and smoking[148, 151] and frequency of attacks.[152]

Ischemic stroke occurring during a migraine attack however is rare and has an estimated prevalence of only 0.5–1%.[153, 154] Migrainous infarction is defined as ischemic stroke developing in tandem with an ongoing migraine attack with prolonged (>60 minutes) aura with evidence of ischemic lesion in relevant vascular territories on neuroimaging.[155]

The presence of a patent foramen ovale (PFO) in which there is communication between the right and left atria, creating a route for paradoxical embolism to the brain and ischemic stroke, is associated with migraine and particularly migraine with aura in a bidirectional manner.[156] Individuals with migraine are more likely to have a PFO, and individuals with a PFO more likely to have migraine. PFO is also associated with cryptogenic stroke,[157] especially in young persons.[65, 156] The mechanisms connecting PFO, migraine and ischemic stroke are not completely clear. The common link may be microembolic noxious particles and airbubbles which have been shown to trigger cortical spreading depression (CSD), suggesting that PFO may cause both ischemic stroke and migraine in some individuals.[158] Microemboli may also be carriers of vasoactive substances such as endothelin or serotonin capable of triggering migraine attacks.[159, 160] Genetic factors contributing to the co-existence of migraine and PFO in some individuals have been suggested.[161] There are also studies with suggestive findings relating to PFO and ischemic stroke in the posterior vascular territory. In one study with young stroke patients, PFO was detected more often in PCiS vs. ACiS patients (PCiS: 31.1%, ACiS: 25.4%, p=0.029),[65] and another study found that PFOs with right-to-left shunting only on provocation was associated with lesion location in the vertebrobasilar rather than carotid circulation (OR=3.31; p=0.03).[162]

Imaging findings in migraine

Accumulated neuroimaging data indicate that infarct-like lesions and white matter abnormalities (WMA) on MRI are more common in migraine patients than in control subjects.[163-166] The association between infarct-like lesions and WMA is stronger for

migraine with aura than for migraine without aura.[167] Migraine-associated infarct-like lesions appear to be more common in the posterior circulation, especially the cerebellum.[73, 163, 168] In the NOMAS study,[169] the association of migraine and infarct-like lesions was significant for migraine without aura, and 10% of the subclinical brain lesions were located in the cerebellum. Several MRI studies indicate that migrainous infarction predominantly occurs in the posterior circulation.[154, 170]

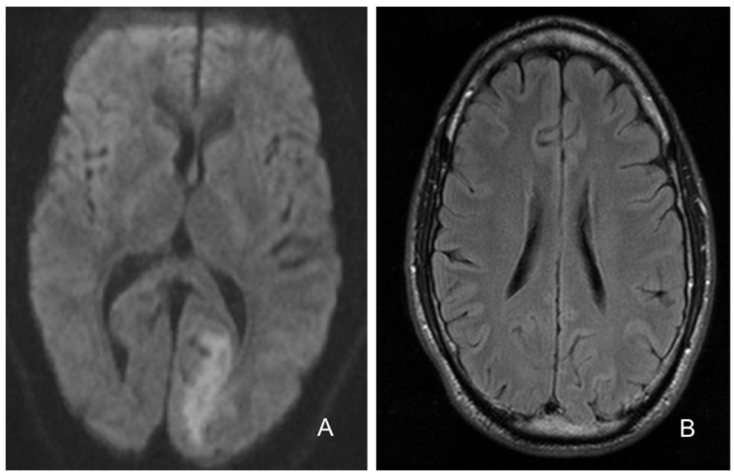

Figure 11.
Brain MRI of a 44 year-old male patient with a history of MA, presenting with right homonymous hemianopsia and migraine headache. (A) MRI-DWI on day 3 showing restricted diffusion in the occipital lobe. (B) On follow-up imaging 18 days later there was no visible ischemic lesion.

Genetics

Shared genetic factors in migraine and ischemic stroke are evident in some rare, monogenic diseases in which both conditions co-exist. One example is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). In this disease, MA is an early symptom and the condition is later complicated by subcortical ischemic lesions and dementia.[171] The disease is linked to mutations in the NOTCH3 gene expressed in cerebral vascular smooth muscle cells.[172] Monogenic diseases do not explain the association between migraine and ischemic stroke generally, but may serve as hypothesis generating models of pathomechanisms influencing stroke risk.

A shared genetic background between migraine and ischemic stroke and stroke subtypes has been demonstrated in GWA studies.[126] Genetic overlap was stronger between MO and ischemic stroke than MA and ischemic stroke. MO also showed greater genetic overlap than MA with the subtypes LAA and CE. The results are not consistent with

epidemiological data supporting a stronger association between MA and stroke. The reason for this may be that rare genetic variants not detected in GWA studies contribute to a greater genetic ischemic stroke risk in MA than in MO. The biological mechanisms associating migraine and ischemic stroke are not understood fully. Potential explanations include CSD during migraine aura followed by hypoperfusion and vasoconstriction predisposing to ischemic injury,[154] endothelial dysfunction,[173, 174] hypercoaguability,[175] and the interaction of lifestyle choices and known cardiovascular risk factors common to both conditions.[159]

Aims

The general aim of this thesis was to investigate risk factor and genetic associations in PCiS compared with ACiS patients in whom the diagnosis of PCiS or ACiS had been definitively established by the presence of acute ischemic lesions on MRI-DWI, and to investigate the prevalence and laterality of a normal variant of the posterior cerebral artery in the same ischemic stroke population compared with an unselected hospital-based population without stroke diagnosis.

The specific aims of studies I–IV were:

I To develop a neuroimaging evaluation protocol, and to use the protocol to phenotype a multicenter ischemic stroke population according to neuroimaging features on MRI-DWI and MRA. Further, to specifically define DWI lesion location as PCiS or ACiS for subsequent use in Study II–IV.

II To investigate vascular risk factor association and ischemic stroke subtypes in PCiS vs. ACiS, and to describe DWI lesion location in relation to ischemic stroke subtype in PCiS.

III To examine potential differences in genetic background and genetic risk contribution in PCiS vs. ACiS by generating migraine polygenic risk scores and comparing them between PCiS and ACiS, and separately for PCiS and ACiS vs. non-stroke control subjects.

IV To determine the prevalence and laterality of FTP configurations in a population of MRI-DWI verified ischemic stroke patients investigated with MRA and compare the findings with an unselected hospital-based population investigated with CTA, and to evaluate the association between FTP and PCA territory infarction.

Methods

Study material and populations

Studies I–IV in this thesis are based on data collected from ischemic stroke patients at 12 centers in the US (5) and Europe (7). The centers are participants in the National Institute of Neurological Disorders and Stroke Genetics Network (NINDS-SiGN) study.[80] They have contributed neuroimaging, demographic, genotype and ischemic stroke subtype data to the MRI-GENetics Interface Exploration (MRI-GENIE) study.[176] The neuroimaging and associated clinical and genotype data from the 12 SiGN sites in the MRI-GENIE study constitute the core study material of this thesis.

Genetic and phenotype data for migraine patients used in Study III were obtained from previously published and publically available GWAS summary results. The unselected hospital-based population in Study IV was derived from a consecutive sample of CTAs performed at Skåne University Hospital in Lund.

National Institute of Neurological Disorders and Stroke Genetics Network (NINDS–SiGN) study

The SiGN study was established by NINDS, a part of the U.S. National Institute of Health (NIH), to facilitate genetic associations studies on ischemic and hemorrhagic stroke by multicenter collaborations. Such studies require large genotype datasets and consistent assignment of ischemic stroke etiology, achievable only through large-scale collaborative efforts. SiGN consists of 24 designated Genetic Research Centers (GRC) in the US (13 centers) and Europe (11 centers) with DNA samples, clinical data and neuroimaging of IS patients. Led by a Scientific Steering Committee, the study has four cores. The Imaging core is the central repository for clinical MRIs submitted by the GRCs.

Ischemic stroke subtypes in SiGN

Patients in the SiGN study are phenotyped according to the causative classification of stroke (CCS) system.[78] Local adjudicators worked to harmonize the phenotyping of ischemic stroke cases across SiGN sites with a web-based, semiautomated system facilitating assignment of the most likely causative mechanism.[78] The CCS system takes into account multiple diagnostic modalities (perfusion- and/or diffusion-weighted MRI, CTA and MRA of extracranial and intracranial arteries and echocardiography) in a standardized approach to assign likely causative subtypes. Interrater reliability for the system in an international multicenter study was high (Kappa 0.80) when 20 raters applied the system to the same 50 consecutive cases.[79]

Genotype data

Patients were genotyped at the original site and the genotype data submitted to SiGN, or they were genotyped within the SiGN study at the Center for Inherited Disease Research in Baltimore. A detailed description of the genotyping methods within SiGN has been published.[80] AIS patients with a determined CCS subtype, i.e. not assigned the subtype "Undetermined" were prioritized for genotyping. Known rare causes of stroke were excluded. Ischemic stroke cases that are CCS classified as cryptogenic but have been adequately evaluated according to the CCS algorithm were included.

The MRI-Genetic Interface Exploration (MRI–GENIE) Study

The MRI-GENIE study[176] was founded in 2014. Its aim is to discover the genetic contribution to cerebrovascular disease and acute ischemic stroke through analysis of neuroimaging phenotypes on brain MRIs in conjunction with clinical phenotype, ischemic stroke subtype and genotype data.[176] SiGN centers were invited to participate in the study by submitting clinical MRI scans of AIS patients.

As of March 2017, twelve of the 24 SiGN sites had contributed 3 301 scans of clinical IS patients. Today, (2021) there are 23 contributing SiGN sites and >9 500 available clinical brain MRIs. As part of the study protocol, participating SiGN sites provided phenotypic data (SiGN ID, age, sex, race, ethnicity, and infarct location). Conventional vascular risk factor data (hypertension, diabetes mellitus, atrial fibrillation, coronary artery disease, and smoking status) were collected at the individual study sites at the time of patient enrolment.

An overview of the 12 SiGN sites contributing to the MRI-GENIE study is provided in Table 5. All patients with available MRI scans from the 12 sites were eligible for inclusion in this thesis.

Table 5.
SiGN centers contributing MRI data to the MRI-GENIE study.

Study Name	Center and Location	Study design	Population	Special inclusion
BASICMAR– BASe de datos de ICtus del hospital del MAR	IMIM-Hospital del Mar– Barcelona, Spain	Hospital-based	First-ever or recurrent acute stroke	
BRAINS– Bio-Repository of DNA in stroke	Imperial College– London, England	Hospital-based	First-ever or recurrent acute stroke	
GASROS– Genes Affecting Stroke Risk and Outcome Study	Mass General Hospital– Boston, USA	Hospital-based	First-ever or recurrent acute stroke	
GCNKSS– Greater Cincinnati/Northern Kentucky Stroke Study	University Cincinnati– Greater Cincinnati region, USA	Population-based	First-ever or recurrent acute stroke	
GEOS– Genetics of Early Onset Stroke	University Maryland– Greater Baltimore, USA	Population-based	First-ever ischemic stroke, acute or identified <3 years	<49 years of age
SAHLSIS– Sahlgrenska Academy Study on Ischemic Stroke	Sahlgrenska Hospital– Gothenburg, Sweden	Hospital-based	First-ever or recurrent acute stroke	<70 years of age
GRAZ	Medical University– Graz, Austria	Hospital-based	First-ever or recurrent acute stroke	
ISGS– Ischemic Stroke Genetics Study	Mayo Clinic– multicenter, USA	Hospital-based	First-ever ischemic stroke cohort	
KRAKOW	Jagiellonian University– Krakow, Poland	Hospital-based	First-ever or recurrent acute stroke	
LEUVEN	University Hospitals– Leuven, Belgium	Hospital-based	First-ever and acute recurrent stroke	Image verified ischemia
LUND– Lund Stroke Register	Lund University Hospital– Lund, Sweden	Hospital-based	First-ever acute stroke	
MIAMISR– Miami Stroke Registry and Biorepository	University Miami– Miami, USA	Hospital-based	First-ever or recurrent acute stroke	

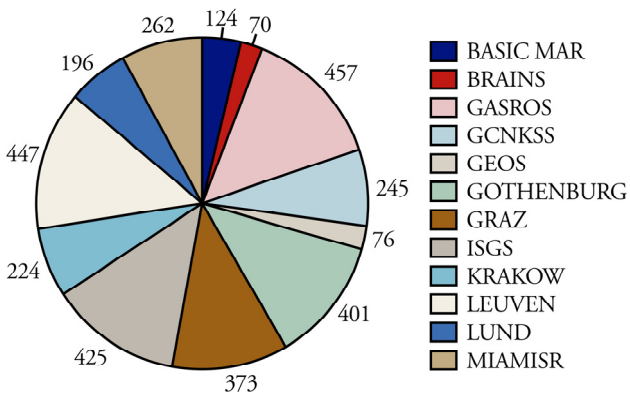

Figure 12.
Number of scans contributed to the MRI-GENIE study per site.

Methods and procedures

Stroke phenotyping

We developed a structured protocol to evaluate and categorize the data in the MRI-GENIE neuroimaging repository for use in subsequent studies. The study protocol contained imaging data variables for predefined categories of anatomical location, arterial supply areas, major arterial vascular territory, and vascular segments.

MRI-DWI sequences were assessed regarding the presence of acute ischemic lesions, anatomical location, side of lesion, single or multiple lesions, and subcortical/cortical location. MRA data were assessed regarding vessel anatomy and variants, patency, and site of occlusion.

Images submitted to and deposited in the registry erroneously, such as computed tomography studies or imaging of anatomical regions other than the brain and cerebral vasculature were excluded from all further analyses.

The study protocol is reproduced in detail in the Appendix section (App. I).

Neuroimaging findings and patient characteristics in a multicenter IS population (I)

Study I is a retrospective cross-sectional and descriptive baseline study of the neuroimaging and associated ischemic stroke phenotype data in the MRI-GENIE repository. We used our study protocol to assign the MRI-DWI and MRA data to predefined categories of anatomical lesion location, vascular territory and major arterial supply area.

Two licensed neuroradiologists (JW, MD) at Skåne University Hospital, Lund, reviewed all MRI scans (3 301 correctly included) in the repository. Deidentified images were available for review through a secure computer viewer. The original neuroimaging data contained the local radiology lab report on anatomical lesion

location; right/left hemisphere, cerebellum, or brainstem. To avoid bias in the interpretation of image data the neuroradiologists were blinded to these data, as well as stroke subtype and other clinical data from the source centers. Findings according to the predefined variables in the structured protocol were entered into pre-prepared data sheets for each of the twelve study sites. Lesion locations on DWI were recorded as cortical and/or subcortical, single/multiple and lacunar/non lacunar for supratentorial lesions. Single subcortical lesions visually gauged to be smaller than 15 mm were defined as lacunar. Lesion laterality was also recorded. Assignment of anatomical lesion location and vascular segments were performed using a widely used map of the major vascular territories. A version of the map can be found in the Introduction.

Vessel patency was evaluated regarding stenosis and occlusion on MRA images. Stenosis was assigned when the visually gauged luminal reduction was >50%.

Vascular risk factors and etiology in PCiS vs. ACiS (II)

In study II we investigated vascular risk factor association and ischemic stroke subtypes in PCiS vs. ACiS, and DWI lesion location related to CCS subtype in PCiS based on the MRI/MRA data characterized in study I.

Patient selection

After reviewing the 3 301 scans in the image repository we included 2 469 patients with visible DWI lesions on MRI.

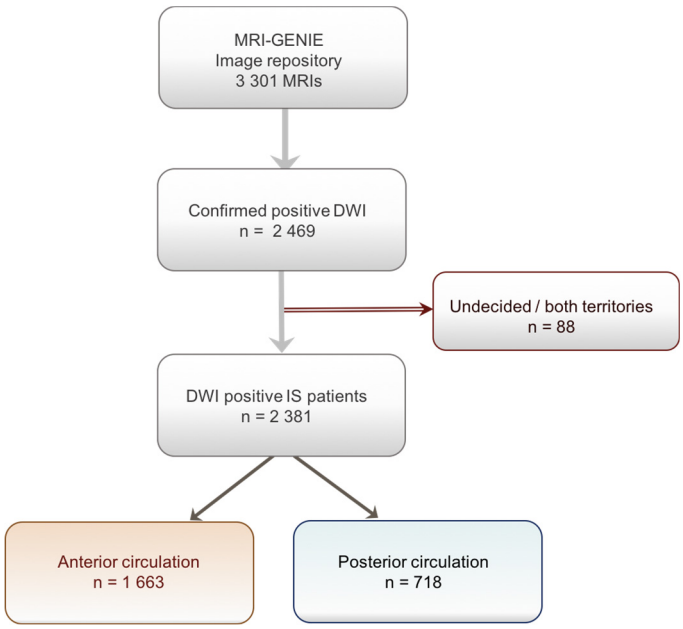

Figure 13.
Flow chart of patient selection in study II

ACiS was defined in patients with DWI lesions in the supply areas of the MCAs and the ACAs and PCiS was defined in patients with DWI lesions in the VB and PCA supply areas. Thalamic and inferomedial temporal lobe DWI lesions were considered posterior. We included thirteen patients with PCA territory infarcts in the occipital lobe and ipsilateral FTP as posterior circulation strokes, because their image phenotype corresponded to posterior stroke.

NIHSS was not available as a phenotype variable in the MRI-GENIE dataset. For the analysis of NIHSS in study II, we requested NIHSS data for the SiGN sites included in this thesis through the Genetics of Ischaemic Stroke Functional Outcome (GISCOME) study.[177]

Genetic overlap between migraine and PCiS vs. ACiS (III)

In study III we examined genetic overlap between migraine and DWI verified PCiS. We also investigated if genetic overlap differed between PCiS and ACiS, and between PCiS and ACiS separately compared to non-stroke control subjects.

We constructed migraine polygenic risk scores for three clinical migraine phenotypes using summary statistics from migraine GWA studies. For detailed descriptions of genotyping data analysis strategy, genotyping platform characteristics, and quality control methods within SiGN/MRI-GENIE, please see the Methods section of Paper III as appended in this thesis, and the original publications in which these methods are described in detail.[80, 176]

Genome-wide association data

Background

GWA studies investigate genetic variations, most often single nucleotide base-pair changes, across the human genome to identify genetic risk factors for common diseases in a population. Single nucleotide base-pair changes that are abundant in a population are termed single nucleotide polymorphisms (SNPs). SNPs represent the most common variant in the human genome. The common disease/common variant hypothesis states that the genetic risk of common diseases is conferred by genetic variation that is very common in the population. This means that the effect size, i.e. influence on disease risk of each individual SNP identified as associated with a disease in GWA studies is very small. It also means that many risk SNPs (alleles) must be involved in influencing the genetic risk of common disorders.[112] In line with this hypothesis, common, complex disorders are therefore termed polygenic in terms of the genetic influence on disease risk and phenotypic trait.

Because of the small effect size of common variants on complex disorders, very large study samples are needed in GWA studies to achieve statistical power to detect associations. GWA study summary statistics are available from several sources, such as supplementary files, links or references in a published paper, submitted to an online catalog or published on a website through a consortium. Summary statistics for the migraine GWA meta-analysis used in Study III are available at: https://www.ebi.ac.uk/gwas/downloads/summary-statistics

Migraine GWAS data

We used publically available migraine GWAS summary results[127] for three migraine phenotypes—any migraine (M), migraine without aura (MO) and migraine with aura (MA)—to generate individual PRSs in our ischemic stroke cases with PCiS and ACiS. The migraine GWAS was a meta-analysis of 22 migraine GWA studies of 375 000 individuals with 59 674 cases and 316 078 control subjects.[127] Migraine classification in the studies used for the discovery meta-analysis was based on a combination of ICHD-II criteria and self-reporting. In the meta-analysis, the number of cases for the

M phenotype were 59 674, with much fewer cases for MO (8 348) and MA (6 332). All samples in this migraine GWAS were of European genetic ancestry.

Polygenic risk scores

Background

PRSs reflect the additive effect of many individual common variants (SNPs) identified in a previous GWAS as associated with a disease/phenotypic trait. The score is commonly calculated using GWAS summary statistics of the trait/disorder of interest. Technically the score is the sum of the number of risk alleles in an individual (0, 1 or 2) weighted by the effect size estimated in the discovery GWAS.[133] For polygenic disorders (such as IS or migraine), PRSs achieve greater predictive power than a single common genetic variant.

Construction of migraine PRS

The PRSice-219 software package was used to calculate PRSs for the MRI-GENIE study individuals. The migraine-associated variants included in the GWAS summary statistics with p-value thresholds of 10^{-5}–$\leq 10^{-8}$ were clumped to remove SNPs in linkage disequilibrium (LD) with each other. We retained only the SNPs with the lowest GWAS p-value, i.e. with the highest statistical significance, in each LD block. Clumping reduces correlation between SNPs that are kept for further analyses. The weighted beta coefficients were then summed across all SNPs to generate individual PRSs for PCiS and ACiS. The PRSs for PCiS and ACiS cases were used to calculate different p-value levels (10^{-5}–$\leq 10^{-8}$) for each of the three migraine phenotypes M, MO, and MA.

The 10^{-5} level contained all of the retained SNPs, the 10^{-6} level contained those with p-values $<10^{-6}$ and so on.

Since the discovery migraine GWAS had not detected any genome-wide associations for MA at the significance level of $\leq 10^{-8}$, polygenic risk scores for MA did not include SNPs with this level of significance.

Ischemic stroke population (target population)

In study II we had identified 718 PCiS and 1 663 ACiS patients in the MRI-GENIE cohort. Because we could not be sure if patients with DWI lesions in the posterior cerebral artery territory and coexisting ipsilateral fetal posterior cerebral artery had experienced a thromboembolic event from the anterior circulation they were excluded

from this study. Patients with atrial fibrillation were also excluded because we expected that distal emboli should be proportionally equally distributed between the posterior and anterior circulation territories. We also hypothesized that shared genetic background in migraine and PCiS may be more relevant in the ischemic stroke subtypes large artery atherosclerosis and small vessel disease and in cardioembolic stroke of other causes than atrial fibrillation. After stratification based on genetic ancestry and genotyping platforms, the remaining number of patients for the genetic analyses was 505 PCiS and 1 182 ACiS.

Ischemic Stroke Cohort Genotyping

Genome-wide genotyping data were available through SiGN.[80] For the main analyses, we used ischemic stroke cases of European ancestry only (N=1 543, of which 464 PCiS, 1079 ACiS). In the transethnic analyses, we included cases of African ancestry (n=144).

Non-stroke control subjects

Genotype data from 15 396 non-stroke control subjects were available through SiGN. The non-stroke control subjects were matched to stroke subjects on the basis of ancestry and genotyping array. Age data were not available for all control subjects. Quality control of genotype data for all non-stroke control subjects have previously been conducted through SiGN.

Prevalence and laterality of FTP in two patient populations (IV)

Paper IV is a cross-sectional study investigating the prevalence and laterality of a fetal posterior cerebral artery (FTP) in ischemic stroke patients with available MRA in the MRI-GENIE study and an unselected hospital-based population investigated with CTA

Patient selection

Ischemic stroke population

From the MRI-GENIE repository, we included all patients with verified acute ischemic lesions on DWI (n= 2 469). Patients without imaging of intracerebral vessels were excluded. In total 1 407 patients with DWI lesions and available MRA remained for analysis.

Unselected hospital-based population

Consecutive CTA investigations of extra- and intracranial vessels performed at Skåne University Hospital in Lund during a 12-month period in 2016–2017 were reviewed. The referrals were on any indication by primary, hospital and emergency care physicians. Two neuroradiologists and one radiology resident independently reviewed the scans blinded to the initial radiology lab reports. In total 546 consecutive CTA investigations were reviewed. Scans were excluded if artefacts or inferior technical quality made evaluation of target vessels uncertain. After exclusion, 489 CTAs remained for analysis.

Definition of FTP

We used the definition proposed by van Ramt:[26] FTP was assigned in patients in whom the P1 segment was of smaller calibre than the ipsilateral PcomA, or the P1 segment completely lacked connection with the BA. A visible but hypoplastic P1 segment in the presence of a larger PcomA was defined as *partial FTP*. If the P1 segment could not be visualized i.e. there was no luminal connection between the remnant PCA and the BA, a *complete FTP* was identified. Vessel calibres were visually gauged by the reviewers.

Imaging modality

Detection of FTP in the ischemic stroke population was based on MRA from the SiGN/MRI-GENIE collaboration, and in the unselected hospital–based group from Lund on CTA. Compared to "gold standard" conventional angiography (DSA) both modalities have a similar high sensitivity for detecting intracerebral vessel anatomy.[178] A similar study to ours using CTA as reference found the sensitivity and specificity for detecting FTP with MRA compared to CTA (their reference) to be 90.9% and 99.2% respectively.[179]

Statistical methods

SPSS v. 24 (I), 23 and 25 (II, II), and 26 (IV) were used for data processing and analyses. Descriptive statistics including means with standard deviations (SD) for parametric variables and medians with interquartile ranges (IQR) for non-parametric variables were used.

Paper I: Comparison of proportions between groups in the analyses of left vs. right-sided lesions among 1) all patients, and 2) among anterior circulation lesions only were performed using the Chi-Square test. Significance level was set to $p<0.05$. Interrater agreement between the two reviewers was measured using vascular territory as a single review criterion. Agreement was assessed by calculating the weighted and unweighted Cohen κ coefficient.

Paper II: Univariate analysis for each of the two stroke phenotypes and between groups was performed using the Mann-Whitney test for non-parametric variables and the Chi-Square test for categorical variables. We included all vascular risk factors in the univariable analyses. A logistic regression model was created to compare risk factors between groups. Variables that did not differ significantly between groups in the univariable analyses were excluded from the model in the multivariable analysis. Missing data on vascular risk factors were not included in any analyses. Missing data did not exceed 3% for any single variable except NIHSS (22%).

Paper III: In our primary analyses, we tested whether genetic risk of migraine was more strongly associated with PCiS than with ACiS by comparing migraine PRSs for three migraine traits (M, MO and MA) between PCiS and ACiS in European ancestry cases.

In secondary analyses we evaluated if migraine PRSs were differentially associated with PCiS or ACiS by comparing migraine PRSs separately in PCiS and ACiS vs. non-stroke control subjects. We also performed transethnic analyses using both of the approaches described above after including cases of African ancestry in the target group.

Logistic regression analyses for the primary analysis and the secondary analyses, were controlled for age, sex and principal components of genetic ancestries. The results of the logistic regression analysis for each stratum were meta-analyzed assuming fixed effects.

In the primary analyses we applied correction for multiple testing, considering a p-value of 0.016 to be significant based on analyzing three migraine traits (Bonferroni correction). We did not consider post-hoc power calculations necessary to perform since significant p-value results were for PCiS despite our study's higher power for ACiS. The method of using PRSs based on different GWAS p-value thresholds was not corrected for multiple testing due to the complete overlapping sets of SNPs from one GWAS cut-off threshold to the next threshold.

In the meta-analyses combining European and African cases (n=505 PCiS, n=1182 ACiS) we assumed first fixed then random effect without any significant differences between results. Estimated beta coefficients from the logistic regression analyses were

converted to odd ratios and expressed as per one standard deviation increases of the PRSs.

Paper IV: Categorical variables in the analysis of infarct location were compared between groups using the Pearson Chi-Square test. For comparison of FTP lateralization (right vs. left) we performed a binominal test of proportions to evaluate if the proportion of FTP on either side was significantly different from 0.5, positing the null hypothesis that the probability of finding FTP on the right or the left side was the same as that of a coin flip (50/50). Confidence intervals for the binominal test were based on the Clopper-Pearson formula.

Ethical approval

All study protocols regarding human subjects have been approved by their local institutional review board, and written consent was given by all participants or through surrogate authorization at the time of enrolment at the original sites in the SiGN study.

The MRI-GENIE study has been approved by the institutional review board at the Massachusetts General Hospital, Boston, MA (protocol number: 2001P001186).

The collection of anonymized CTA data for study IV has been approved by the Regional Ethical Review Board, Lund University, Sweden: # 2018/411.

Results

Neuroimaging findings and patient characteristics in a multicenter IS population (I)

Study I details patient characteristics and features of stroke lesion distribution in a cross-sectional multicenter sample of patients with clinical stroke. It is not primarily focused on findings related to the posterior circulation.

Available MRI and MRA sequences

As of May 2017, the MRI-GENIE repository contained 3 319 studies submitted by 12 sites within SiGN. The number of available studies and sequences as well as reasons for exclusion from review are shown below.

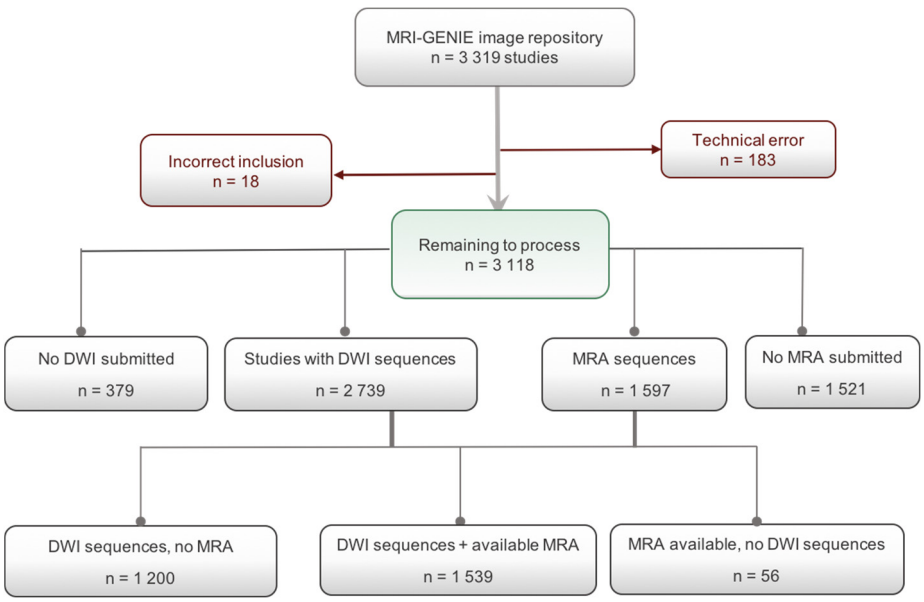

Figure 14.
Chart of available neuroimaging studies and sequences in the MRI-GENIE repository.

The 12 SiGN sites contributed between 70 and 475 patients. Detailed radiology contributions per site are presented in Table 6.

Table 6.
Radiology contributions per site (n).

SITE NAME	Total	Mean age years (SD)	Technical error	No DWI images	Infarct not seen	MRI	DWI	MRA	DWI + MRA
BASICMAR	124	70 (11)	1	6	7	123	117	0	0
BRAINS	70	63 (16)	5	6	24	65	59	17	17
GASROS	457	65 (14)	2	0	34	455	455	238	238
GCNKSS	245	65 (14)	15	21	19	230	209	125	118
GEOS	76	42 (7)	0	10	9	76	66	47	38
SAHLSIS	401	52 (12)	8	191	24	393	202	103	93
GRAZ	373	63 (14)	123	73	15	250	177	72	66
ISGS	425	65 (15)	12	27	37	413	386	307	291
KRAKOW	224	60 (14)	12	32	29	212	180	3	3
LEUVEN	448	67 (15)	4	7	50	445	438	400	395
LUND	196	63 (13)	0	0	17	196	196	53	53
MIAMIS	262	62 (14)	2	6	6	260	254	232	227
TOTAL	**3301**	**62 (14)**	**183**	**379**	**271**	**3118**	**2739**	**1597**	**1539**

Patient characteristics and risk factor distribution

Of the 3 301 patients in the repository, 39% were women. The site with the lowest proportion of women included 26% women; the highest proportion of women among the sites was 47%. Mean age was 62±14 (SD). One site included only young stroke patients aged 15–49 (GEOS) and one site included only patients <70 years of age (SAHLSIS). A majority of patients were "White" (62%), 28% were identified as "More than one race," and 5% as "Black or African-American." Hypertension was the most common vascular risk factor (65%). Demographis and risk factor prevalences are shown in Figure 15.

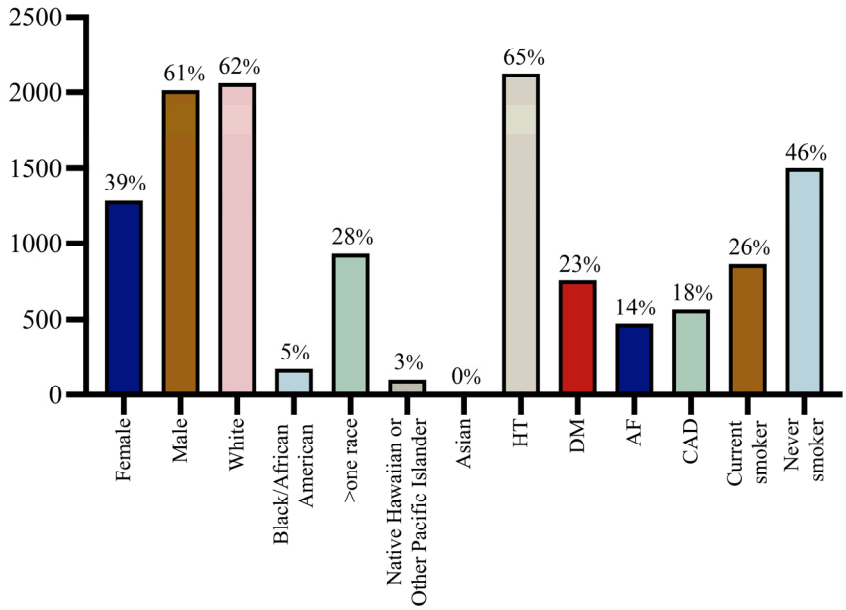

Figure 15.
Demographics and risk factor prevalence in the MRI-GENIE study population (n = 3 300). HT, hypertension; DM, diabetes mellitus; AF, atrial fibrillation; CAD, coronary artery disease.

Etiology

Ischemic stroke subtype classification according to CCS was available for all included patients. A majority of patients (90%) had also been subtyped according to TOAST criteria. There were discrepancies in subtype assignment between CCS and TOAST. The largest difference was for the subtype CE which was assigned to 15% of patients according to CCS and 23% according to TOAST criteria (Fig. 16).

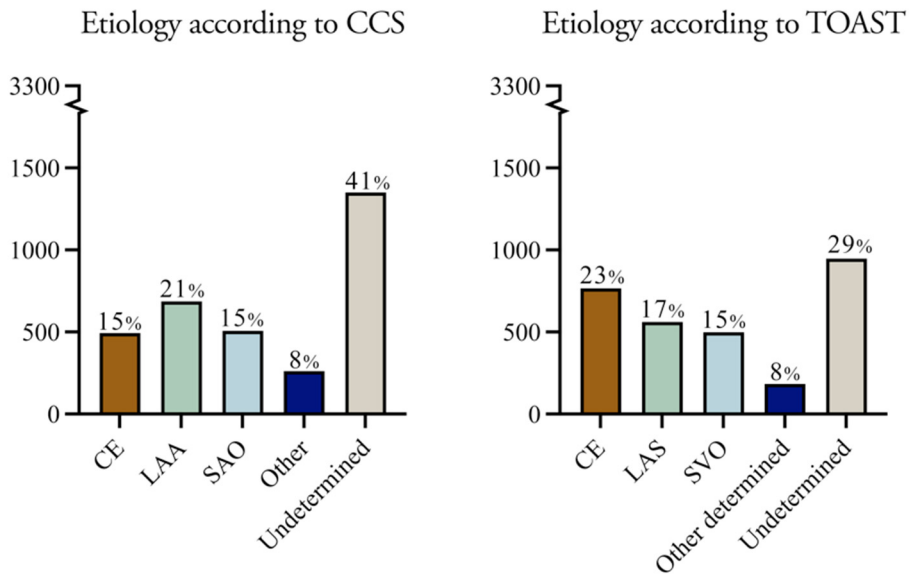

Figure 16.
IS subtype classification according to CCS (n=3 300) and TOAST (n=2 957) for all patients in MRI-GENIE.

MRI–DWI characteristics

Timing of MRI imaging

For the 2 739 patients with available MRI DWI scans, the median time from symptom onset to scan was 1 day. After stratifying time to scan into three time windows (0–48 hours, 2–14 days, and >14 days), we found that 1 227 patients (45%) were scanned within 48 hours. A small portion of patients (4.5%) had MRI-DWI beyond 14 days.

Main findings on stroke lesion localization and topology

The most common stroke lesion location was cortical/subcortical hemispheric (52%). The second most common was deep grey matter (basal ganglia and thalamus) detected in 15% of patients. The anterior circulation territory was involved in 67% of cases and the posterior circulation in 29%. Evidence of lesions in both circulation territories was seen in 4%. The most frequently involved arterial territory was MCA. The VB territory was more frequently involved than the PCA.

There were 2 238 patients with unilateral lesions and those were detected more often in the left vs. right hemisphere (p=0.013) (Table 7). Stratification of lesion side according to arterial distribution resulted in a significant side difference only for supratentorial lesions and finally only for lesions in the MCA territory.

Table 7.
Laterality of lesion burden.

LESION LOCATION	LEFT	RIGHT	BOTH	p-value (left vs. right)
All lesions*	1 188	1 070	228	***0.013***
Supratentorial	963	861	81	***0.018***
Infratentorial	191	185	47	0.757
MCA territory	850	764	102	***0.032***
ACA territory	30	33	14	0.705
PCA territory	131	117	111	0.374
Vertebrobasilar	215	199	147	0.432

* Individual patients may contribute lesions to more than one category, resulting in a total number of lesions exceeding the number of patients in the analysis (n=2 238).

MRA characteristics

The intracranial portions of the VA, BA, PCA, ICA, MCA, and ACA arteries were evaluated on 1 596 MRA scans. Extracranial portions of cerebral arteries were not evaluated. Stenosis was visually gauged and defined as >50% reduction of lumen diameter. The most common sites of moderate to severe vessel stenosis was the BA (4%) and the VA (3%). VA occlusion were present in 12%. (Figure 17).

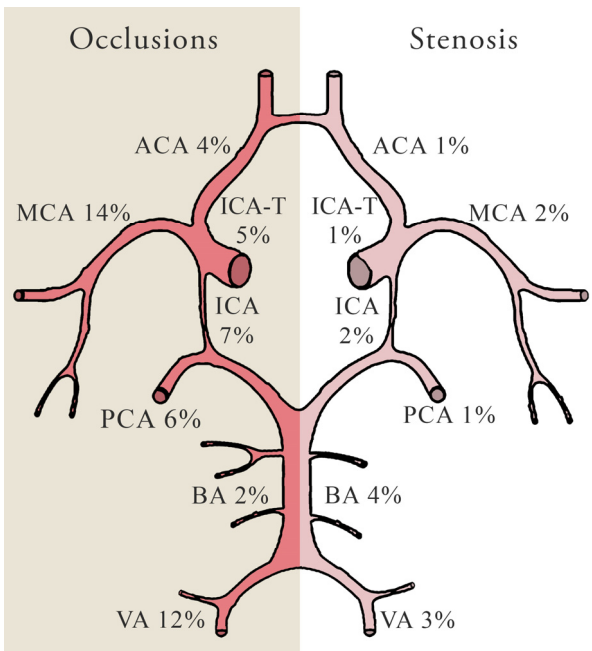

Figure 17.
Percentage of stenosis and occlusions per vessel site in 1 596 evaluated MRAs. Each half of the illustration refers to all occlusions and stenotic vessels, irrespective of side (right/left).

Vascular risk factors and etiology in PCiS vs. ACiS (II)

We identified 2 469 patients with visible DWI lesions on MRI-DWI from the MRI-GENIE repository. Patients were defined as ACiS or PCiS according to lesion location on DWI. Exclusion criteria were DWI lesions in both vascular territories (n=87). One (1) patient was excluded because vascular territory could not be determined. In total, 2 381 patients were included.

Demographics and vascular risk factor distribution in PCiS vs. ACiS

We found 718 (30%) patients with PCiS and 1 663 (70%) with ACiS. There were significant differences between the vascular territories in age, sex and risk factor distribution. PCiS patients were younger (62 ±15 PCiS vs. 64 ±15 ACiS) and a larger proportion of patients with PCiS were male (68% PCiS vs. 58% ACiS).

Hypertension was an equally common risk factor in both territories whereas diabetes mellitus was more common in PCiS than in ACiS. Atrial fibrillation was significantly more common in patients with ACiS than PCiS. In the multivariable logistic regression analysis of risk factor association with PCiS vs. ACIS, male sex and diabetes mellitus were independently associated with PCiS (Table 8).

Table 8.
Vascular risk factor association in PCiS vs. ACiS

Vascular risk factors	ACiS n=1 663	PCiS n=718	Univariable		Multivariable[a]	
			OR	95% CI	OR	95% CI
Age (mean, SD)	64±15	62±15	0.99	0.98–0.99	0.99	0.98–1.00
Male (%)	967 (58)	487 (68)	1.52	1.26–1.82	1.46	1.21–1.78
Hypertension[b]	1 096 (66)	451 (64)	0.90	0.75–1.08	–	–
Diabetes mellitus	373 (23)	190 (27)	1.27	1.03–1.55	1.26	1.02–1.56
Atrial fibrillation	261 (16)	78 (11)	0.64	0.48–0.83	0.70	0.52–0.93
CAD[b]	298 (18)	115 (16)	0.88	0.69–1.11	–	–
Current smoking	462 (28)	154 (21)	0.71	0.58–0.87	0.63	0.51–0.79

[a]Logistic regression model adjusting for age, sex, diabetes mellitus, atrial fibrillation and current smoking
[b]Hypertension and CAD were not included in the multivariable analysis

Ischemic stroke subtypes in PCiS vs. ACiS

All patients had been previously subtyped according to CCS within SiGN. The proportion of patients assigned the CCS subtype *Undetermined* was the same in ACiS and PCiS (38% ACiS; 39% PCiS).

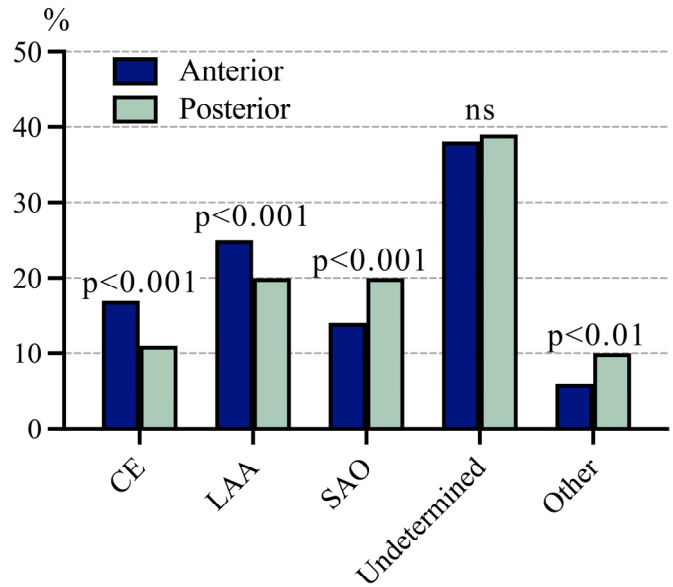

Figure 18.
Proportion (%) of CCS subtypes in PCiS and ACiS patients in MRI-GENIE

A small proportion of patients had been assigned the CCS subtype *Other* in both PCiS and ACiS, though the subtype was significantly more common in PCiS vs. ACiS (Fig. 18). In PCiS the subtypes LAA and SAO were each assigned in 20% of patients. In ACiS, LAA was the most frequently assigned subtype. CE was assigned in 17% ACiS vs. 11% PCiS.

Lesion location and CCS subtypes in PCiS

SAO was assigned in 44% of patients with lesions isolated to the brainstem and in none of the patients with acute lesions in both cerebellum and the PCA territory. Patients with isolated brainstem lesions were assigned *Undetermined* less often than others. LAA was determined to be the causative etiology in 25% of patients with multiple lesions confined to the brainstem. The distribution of CCS types according to lesion location is shown in Figure 19.

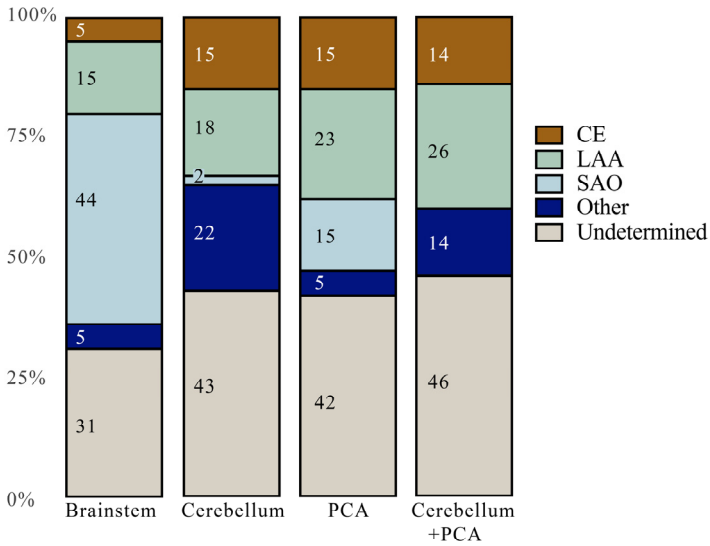

Figure 19.
Lesion locations related to CCS subtype assignment in PCiS patients in MRI-GENIE.
CE, cardioembolism; LAA, large artery atherosclerosis; SAO, small artery occlusion; PCA, posterior cerebral artery.

Genetic overlap between migraine and PCiS vs. ACiS (III)

Study populations

Migraine population

We used the published migraine GWAS meta-analysis which included 375 000 migraine patients, where a total of 8021 SNVs reached p-values of $\leq 10^{-5}$. The number of SNVs included for each migraine phenotype were 7 208 (M), 1 173 (MO), and 464 (MA).[127]

Ischemic stroke target population

In our study, a total of 505 subjects with PCiS and 1 182 with ACiS remained for genetic analyses after internal QC. Of the total number of PCiS and ACiS qualified for genetic analyses, there were 464 PCiS subjects and 1072 ACiS subjects of European ancestry kept for the primary analyses. (Table 9).

Table 9.
Baseline characteristics of ischemic stroke target population

		PCIS N=505 (%)	ACIS N=1182 (%)	P-VALUE
AGE (MEAN, SD)		60.9±14.5	63.2±14.5	0.003*
MALE		342 (67.7)	721 (61.0)	0.009†
DIABETES MELLITUS		133 (26.4)	265 (22.4)	0.081†
HYPERTENSION		313 (62.0)	772 (65.3)	0.207†
CAD		74 (14.6)	186 (15.7)	0.555†
CCS (5 ITEM)				<0.001†
	CE	21 (4.0)	73 (6.2)	
	LAA	106 (21.0)	338 (32.8)	
	OTHER	53 (10.5)	77 (6.5)	
	SAO	116 (23.0)	188 (15.9)	
	UNDETERMINED	209 (41.4)	506 (42.8)	
GENETIC ANCESTRY				0.307†
	EUROPEAN	464 (91.9)	1 079 (91.3)	
	AFRICAN AMERICAN	41 (8.1)	103 (8.7)	

PCiS, posterior circulation ischemic stroke; ACiS, anterior circulation ischemic stroke; CAD, coronary artery disease; CCS, causative classification of stroke; CE, cardioembolic (patients with AF not included in this study); LAA, larger artery atherosclerosis; SAO, small artery occlusion.

*T-test; †Chi-square test

Results of primary and secondary analyses

In the primary analyses we performed meta-analyses of migraine mean PRSs for PCiS vs. ACiS for three migraine phenotypes M, MO and MA. The results of the primary analyses in the European only population are shown in Figure 20. In the secondary analyses of the European population, we compared the association of migraine PRSs with non-stroke control subjects for PCiS and ACiS separately (Table 10).

Any Migraine (M)

Primary analyses–PCiS vs. ACiS

PRSs for the M phenotype across GWAS p-value thresholds from 10^{-6} to 10^{-8} were significantly higher in PCiS compared to ACiS (p=0.001–0.010, OR=1.16–1.20) even after Bonferroni correction. The corresponding difference using the PRSs of M constructed from SNVs with GWAS p-values $\leq 10^{-5}$ remained consistent (OR=1.12) but not statistically significant (p=0.057) before Bonferroni correction.

Secondary analysis–PCiS and ACiS separately vs. non-stroke control subjects

PRSs for the M phenotype at GWAS p thresholds 10^{-5}, 10^{-6} and 10^{-7} were associated with increased risk of PCiS (OR=1.13–1.15, p=0.011–0.03). M derived PRSs at

GWAS p thresholds $10^{-6}$and 10^{-7} showed a decreased risk for ACiS (OR=0.91-0.93, p=0.010–0.039) compared with PCiS.

Migraine without aura (MO)

Primary analyses–PCiS vs. ACiS

PRSs for the MO phenotype were significantly higher in PCiS than in ACiS at the cutoff levels of $\leq 10^{-5}$, $\leq 10^{-7}$and $\leq 10^{-8}$ with association p-values of 0.032–0.048 (OR =1.25–1.26), which is nominally significant ($p<0.05$) but did not meet the strict Bonferroni corrected p-value cutoff ($p<0.016$).

Secondary analysis–PCiS and ACiS separately vs. non-stroke control subjects

PRSs for the MO phenotype were significantly associated with PCiS compared with non-stroke control subjects with association p-values of 0.008 and 0.028 (OR=1.12–1.15), respectively for the PRSs at GWAS p-value thresholds 10^{-5} and 10^{-7}. MO derived PRSs showed no significant association with the risk of ACiS compared to non-stroke control subjects.

Migraine with aura (MA)

In the primary analyses, PRSs derived from MA did not differ between the two vascular territories at any cutoff level. In our secondary analyses comparing vascular territories separately to non-stroke control subjects, PRSs for MA showed no significant association with the risk of PCiS or ACiS at any of our pre-specified GWAS p-value cutoff thresholds.

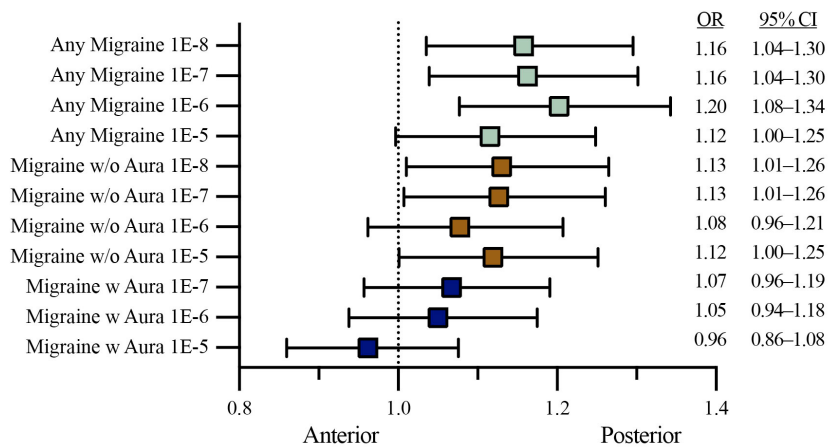

Figure 20.
Migraine PRSs and risk of posterior vs. anterior circulation ischemic stroke. Estimated beta coefficients converted to odds ratios and expressed as per one SD increase of the mean PRS.

Table 10.
Migraine PRSs in PCiS and ACiS separately vs. non-stroke control subjects: European ancestry samples

MIGRAINE PHENOTYPE	GWAS p cutoff	PCiS p	PCiS OR	PCiS 95% CI	ACiS p	ACiS OR	ACiS 95% CI
Any Migraine	1E-08	**0.081**	*1.10*	0.99–1.22	0.064	*0.94*	0.87–1.00
	1E-07	**0.030**	*1.13*	1.01–1.25	**0.039**	*0.93*	0.86–1.00
	1E-06	**0.011**	*1.15*	1.03–1.28	**0.010**	*0.91*	0.85–0.98
	1E-05	**0.023**	*1.13*	1.02–1.26	0.440	*0.97*	0.91–1.04
Migraine without Aura	1E-08	0.069	1.10	0.99–1.23	0.913	1.00	0.94–1.08
	1E-07	**0.028**	1.12	1.01–1.25	0.381	1.03	0.96–1.11
	1E-06	0.091	1.10	0.99–1.22	0.348	1.03	0.96–1.11
	1E-05	**0.008**	1.15	1.04–1.28	0.600	1.02	0.95–1.09
Migraine with Aura	1E-07	0.212	1.07	0.96–1.18	0.514	1.03	0.95–1.11
	1E-06	0.152	1.08	0.97–1.19	0.271	1.04	0.97–1.12
	1E-05	0.916	1.01	0.91–1.12	0.051	1.07	1.00–1.15

GWAS; genome-wide association study; PCiS, posterior circulation ischemic stroke; ACiS, anterior circulation ischemic stroke; OR, odds ratio; CI, confidence interval

Transethnic analyses

Transethnic samples of European and African ancestries were meta-analyzed using the same approaches as in the above primary and secondary analyses.

Primary analyses–PCiS vs. ACiS

PRSs derived from M were significantly associated with PCiS (p=0.001–0.012) compared with ACiS for migraine GWAS p-value cutoff $\leq 10^{-6}$–$\leq 10^{-8}$. We found borderline significance for MO PRSs in PCiS compared to ACiS (p=0.05–0.09). However, the effect size and direction remained comparable to the European ancestry analyses.

Secondary analyses–PCiS and ACiS separately vs. non-stroke control subjects

M PRSs were significantly associated with an increased risk of PCiS only at the GWAS p-value threshold 10^{-6} while the risk of ACiS was decreased. Other thresholds showed no association for M PRSs when compared separately in PCiS and ACiS to non-stroke control subjects. MO PRSs showed an even more prominent association with PCiS than in the European only analyses. There was a significant association with the risk of PCiS at all GWAS p thresholds (10^{-5}–10^{-8}, p-values 0.01–0.04, OR=1.10–1.13). MO PRSs were not significantly associated with the risk of ACiS compared with non-stroke control subjects. MA PRSs showed no differential association with PCiS vs. ACiS and no significant association with PCiS or ACiS when compared to non-stroke control subjects at any cutoff level for the polygenic score.

Prevalence and laterality of FTP in two patient populations (IV)

In this cross-sectional study of FTP prevalence and laterality we compared an IS population investigated with MRA from the SiGN/MRI-GENIE collaboration with an unselected hospital-based population investigated with CTA from Skåne University Hospital, Lund.

Prevalence of FTP

Ischemic stroke population (MRA)

In the IS population (n=1 407), any type of FTP was present in 443 (31%) patients. The most common finding was a unilateral right-sided pFTP (10% of all patients). A unilateral cFTP was detected in 99 patients (7%).

Hospital-based unselected population (CTA)

In the CTA population (n=489) any FTP was detected in 156 (32%) patients. As in the IS MRA population, a unilateral right-sided pFTP was the most common finding. It was detected in 73 (15%) of the patients. Unilateral cFTP was detected in 43 (9%) patients. Table 11 presents the prevalence of FTP configurations in the two study populations.

Table 11.
Prevalence of FTP configurations in the IS population (MRA) and the unselected hospital-based population (CTA)

FTP VARIANTS	MRA N = 1407 (%)	CTA N = 489 (%)
NONE	964 (68)	333 (68)
ANY FTP	443 (31)	156 (32)
BILATERAL	122 (9)	40 (8)
UNILATERAL	321 (23)	116 (24)
PARTIAL FTP	222 (16)	73 (15)
COMPLETE FTP	99 (7)	43 (9)
RIGHT-SIDED*	206 (15)	74 (15)
PARTIAL FTP	146 (10)	49 (10)
COMPLETE FTP	60 (4)	25 (5)
LEFT-SIDED*	115 (8)	42 (8)
PARTIAL FTP	76 (5)	24 (5)
COMPLETE FTP	39 (3)	18 (4)

FTP, fetal-type posterior cerebral artery; MRA, magnetic resonance angiography; CTA, computed tomography angiography; cFTP, complete FTP; pFTP, partial FTP. *Bilateral FTP excluded.

Laterality of FTP

There was a significant difference in the proportion of patients with FTP in the right vs. left hemisphere in both study populations as shown in the table below.

Table 12.
Preferential lateralization of FTP to the right hemisphere in IS patients and an unselected hospital-based population.

	RIGHT FTP	LEFT FTP	PROPORTION RIGHT FTP†	P-VALUE*
MRA	206	115	0.642 (95%CI 0.587–0.694)	<.001
CTA	74	42	0.638 (95%CI 0.544–0.725)	<.005

*Binominal test of proportions. †Confidence interval based on the Clopper-Pearson formula.

Ischemic stroke population (MRA)

In the IS population, FTP was found in the right hemisphere in 206 (15%) patients *vs.* 115 (8%) patients in the left hemisphere. A pFTP was more common than a cFTP regardless of laterality. Bilateral FTP was present in 122 (9%) patients.

Hospital-based unselected population (CTA)

FTP was found in the right hemisphere in 74 (15%) patients *vs.* 42 (8%) in the left hemisphere. A pFTP was more common than cFTP regardless of laterality in this population as well. Bilateral FTP was found in 40 (8%) of patients.

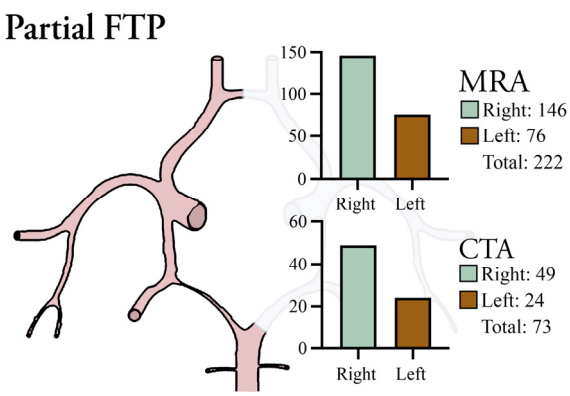

Figure 21.
Number of right vs. left side pFTP in the IS population (MRA) and the unselected hospital-based population (CTA). The P1 segment is hypoplastic but not absent. Only one half of the CoW is depicted here.

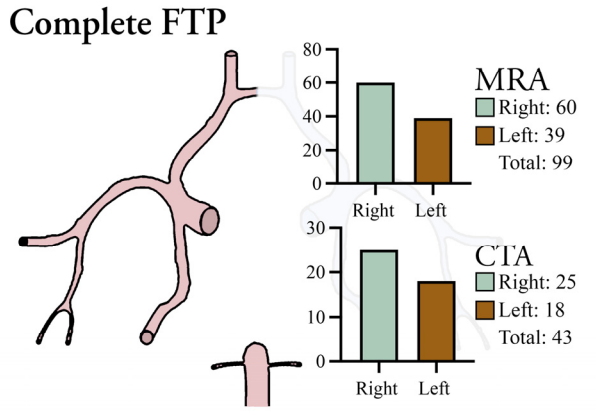

Figure 22.
Right vs. left side cFTP in the IS (MRA) and unselected hospital-based (CTA) populations. The P1 segment is absent and there is no connection with the BA. Only one half of the CoW is depicted here.

Sex differences

There was no difference between women and men in the proportions of FTP (33% vs. 30%) in the IS MRA population. In the CTA population there was a higher proportion of women (37%) than men (27%) with any type of FTP (p=0.03). The proportion of bilateral FTP was higher in women than in men in both the ischemic stroke population (Fig. 23) and the CTA population The sex difference was statistically significant in the IS MRA population (p=0.02, OR 1.5, 95% CI) but not in the CTA population.

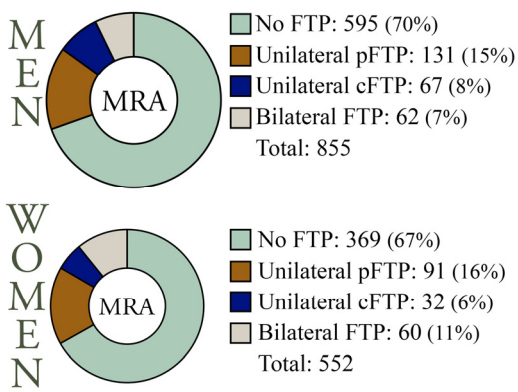

Figure 23.
Sex differences in FTP configuration in the IS MRA population.

Influence of FTP on vascular territory involvement

We analyzed PCA lesion location in relation to the presence of FTP. Patients with no supratentorial lesions (n=201) or bilateral supratentorial stroke (n=125) and remaining patients with bilateral FTPs (n=110) were excluded. There were 971 patients with unilateral, supratentorial ischemic lesions in either hemisphere remaining for analysis. The lesions were in the MCA, ACA, PCA or a combination of territories. There were 506 left hemispheres and 464 right hemispheres to investigate for the presence of FTP and vascular territory involvement. The proportion of patients with ipsilateral FTP to their stroke side was 71/464 (right hemisphere) and 51/507 (left hemisphere). The presence of ipsilateral FTP to stroke side was not associated with PCA territory involvement compared to patients without FTP (p=0.89, 95% CI 0.54–1.71).

Discussion

Discussion of results

Neuroimaging findings and patient characteristics in a multicenter ischemic stroke population (I)

This study presents the imaging findings and associated patient characteristics in an AIS population from 12 study sites in the US and Europe. Describing and characterizing the radiological findings in these patients according to our predefined protocol outlined in the General Methods section was prerequisite work for the subsequent studies in paper II–IV.

PCiS are reported to occur in 16–30% of ischemic stroke patients.[12, 13, 180] Our findings are in the upper range and may be related to difficult–to–diagnose patients being preferentially selected for MRI imaging, leading to sample over representation. On the other hand, a portion of PCiS patients have negative DWI scans initially[107] and would therefore not be included in our sample. Large prospective series of DWI verified ischemic stroke with published data on lesion location according to circulation territory are scarce and true PCiS prevalence in the general population is difficult to state.

Multiple DWI lesions in >1 arterial territory were detected in 11% of patients. TOAST classification for patients with multiple lesions in >1 arterial territory was CE in 33% and LAS in 19%. The prospective NORSTROKE registry[181] which assessed 2 125 DWI positive AIS patients found multiple infarctions in >1 arterial territory in 8.8%. This lesion pattern was associated with cardioembolism and symptomatic ICA stenosis. Other recent studies have confirmed these findings.[76] In our study we were not able to evaluate the extracranial portion of the ICA and consequently not able to relate lesion pattern to ICA stenosis. Multiple lesions in one single arterial territory were very common, detected in 43% of patients. A similar frequency (40%) was found in another study[182], which also observed that the occurrence of new lesions on repeated DWI imaging within 7 days was associated with initial lesion pattern, with the highest risk of new lesions for patients with multiple lesions in >1 territory.

In our study, median time to scan was 1 day (IQR=1–4) for all patients with available DWI sequences with or without lesions (n=2 739). A proportion of patients (12%) with DWI lesions were scanned beyond 28 days from symptom onset. We cannot determine if all lesions represent persisting DWI hyperintensity, or if some are due to stroke recurrence. Early recurrence of DWI lesions even within 1 week of AIS is not uncommon.[183, 184]

We found that acute stroke lesions were significantly more common on the left vs. the right side. On stepwise removal of other arterial territories, the difference remained only for the left vs. right MCA territory (p=0.032). As in our study, left-sided strokes are more common in most hospital-based populations and other studies have dealt with the question of stroke laterality.[185-187] Deficits produced by right-sided lesions are under recognized by patients and physicians, leading to delays in diagnosis and treatment.[186] Biological explanations have been proposed,[187] such as differences in blood flow velocities between the right and left ICA. In terms of stroke laterality, our study population is representative of hospital-based ischemic stroke populations in general.

The mean age of the population is low in comparison with ischemic stroke populations in prospective hospital-based registers. In the Swedish Stroke Register with 89% coverage of 72 Swedish hospitals offering stroke care, the mean age for ischemic stroke patients 2019 was 75.7 (SD12.3).[188] The comparatively young age of our population may reflect a selection of younger patients for MRI investigations in some centers. Young age likely influenced the prevalence of risk factors such as AF which increases with age. The prevalence of AF in our study population (regardless of DWI status, n= 3 301) was 14% which is in line with ischemic stroke populations of the same age range in other studies.

Our multicenter design has advantages compared with single center designs, including the ability to accrue a sufficient sample size of a specific stroke subtype that is more difficult and time consuming to achieve in a single center setting. Multicenter populations are also more likely to be representative of the general population than a single center population. Limitations for this multicentre study are potential differences between individual study sites, such as their use of MRI in clinical practice. Imaging findings could also be influenced by differences in MRI scanners and protocols between centers and over time.

A cardiac source of embolism led to multiple lesions in more than one arterial territory in a third of the patients in our study population. Including MRI-DWI in the workup of patients with a known cardioembolic source is therefore valuable e.g. in revealing ischemic injury that may be masked by clinical deficits such as aphasia. Our study supports previous reports that right-sided stroke is detected less often than left-sided

stroke in the acute setting, which likely reflects an under recognition of right hemisphere deficits and calls for an higher awareness of such symptoms in the clinical setting.

Vascular risk factors and etiology in PCiS vs. ACiS (II)

Patients with detectable DWI lesions in either the posterior or anterior circulation were included (n=2 381) in this study.

PCiS occurred in 30% and ACiS in 70%. One of the included sites (MGH) performs MRI routinely as part of the acute imaging protocol for stroke patients. The site contributed a total of 457 MRI scans of which 421 showed acute DWI lesions. In this subpopulation the prevalence of PCiS was 31%, indicating that our population as a whole may be representative of a hospital-based AIS population.

We found significant differences in the prevalence of traditional vascular risk factors between PCiS and ACiS patients apart from hypertension which was equally common in PCiS as in ACiS. Results from other studies are divergent with some finding hypertension more strongly associated with PCiS vs. ACiS[189, 190] while others do not.[13, 43]

Mean age for PCiS patients in our population was significantly lower than for ACiS. Age difference between these stroke subtypes has not consistently been shown in other studies. Among patients from the study site contributing the sample with the youngest mean age in the MRI-GENIE population (GEOS, n=57, mean age 42±7), PCiS occurred in 36% but this subgroup did not influence the overall results for mean age or PCiS prevalence in the study population.

Age plays a role in defining differences between PCiS and ACiS as it influences stroke mechanisms and risk factors in younger stroke patients. In a large cohort study of patients 18–55 (median 46.5), patent foramen ovale (PFO) and cervical artery dissection were independently associated with PCiS vs. ACiS.[65] The association between PFO and preferential embolism to the posterior circulation has been reported in other studies.[162, 191] Despite the young age of the participants in the above mentioned cohort study, a trend toward higher prevalence of diabetes mellitus was observed in the PCiS group.[65]

In our study, diabetes mellitus emerged as an independently associated risk factor for PCiS vs. ACiS in the multivariable analysis using a logistic regression model adjusted for age, sex and vascular risk factors (OR 1.26; 95% CI 1.02–1.56). Results from other studies also indicate a risk factor association with PCiS in diabetic patients.[7, 13, 45, 192, 193] A multicenter study based on a large hospital stoke registry recently showed measures of glycemic control (plasma glucose concentrations and haemoglobin A1c) to be associated with PCiS compared with ACiS.[194] The consequences of diabetes mellitus

damage the vessel wall endothelium and causes angiopathy through complex pathways.[195] It is plausible therefore, that endothelial function in the vertebrobasilar arterial system is affected differently or earlier by diabetes related injury than those in the carotid arterial system. The stronger association of diabetes mellitus with PCiS vs. ACiS is thus far an observational finding since no longitudinal cohort studies establishing a causative link exist. However, based on accumulated data on well-defined PCiS populations, it seems well worth exploring diabetes as a modifiable risk factor of particular significance in PCiS.

We compared ischemic stroke subtypes according to CCS in PCiS vs. ACiS and analyzed lesion location in relation to CCS subtype in PCiS. The difference between PCiS and ACiS in subtype allocation was significant for all subtypes except the Undetermined category. Large artery atherosclerosis (LAA) and small vessel occlusion (SVO) were the most common causative mechanisms in PCiS, each assigned in 20% of patients. Cardioembolism (CE) was significantly more common in ACiS than in PCiS reflecting the higher frequency of atrial fibrillation in the ACiS group (16% ACiS vs. 11% PCiS). In our study population CE was assigned in both stroke types in relatively few patients compared with the New England medical center posterior circulation stroke registry (NEMC-PCR)[11] (80% MRI verified PCiS, average age 60.5 years). In NEMC-PCR, which is a large and detailed study on PCiS patients, embolism with a cardiac source was determined to be causative in 24% PCiS and 38% ACiS. The reason for the discrepancy between otherwise comparable groups of PCiS patients may be due to different definitions of cardioembolic stroke etiology.

In general, the lack of unity and homogeneity regarding diagnostic criteria for PCiS vs. ACiS and the variation in subtype classification systems in published literature make direct comparisons of proportions of stroke mechanisms in the two vascular territories difficult. A limitation in the present study is the high percentage (39%) of patients assigned Undetermined according to CCS. In the web-based CCS 5 item classification system Undetermined is a heterogeneous group including cases with cryptogenic embolism, competing etiologies and incomplete diagnostic work up.[78] One issue in our study is that the Undetermined group likely contains a high number of patients in whom the evaluation was deemed incomplete. It is possible that this influences the proportion of patients assigned SAO vs. LAA, in particular in PCiS as differentiating between LAA and SAO in PCiS requires visualization of the vertebrobasilar arteries, and determination of infarction size. PCiS patients in whom the evaluation of atherosclerotic vessel sites was limited to the carotid arteries or not performed the evaluation would be incomplete resulting in a subtype assignment of Undetermined.

In Study I which included all patients regardless of DWI lesion status the subtypes SAO (CCS) and SVO (TOAST) were 15% for both classification systems. In this study (II)

including only patients with confirmed DWI lesions the findings were the same, with an equal proportion of patients assigned SAO (15%) and SVO (14%). In contrast, a study specifically investigating the agreement between the two classification systems in the SiGN population (n=13 596) found an overall moderate agreement (kappa =0.59, 95% CI 0.58– 0.60), with the lowest agreement for the SAO/SVO subtype (kappa = 0.56) and the highest for LAA (kappa = 0.71). It is not obvious why CCS/TOAST agreement for SVA/SVO is much higher in our population, but it may be a result of the inclusion of only MRI investigated patients in our study. MRI imaging of the brain in the diagnostic workup has been shown to increase agreement between the CCS and TOAST classification systems for the subtype SAO[81] and it is also possible that patients selected for MRI-DWI already had been investigated with CTA.

Relating DWI lesion location and pattern to causative mechanism we found that solitary lesions in the brainstem were very strongly associated with the SAO classification (OR 9.9, 95% CI 6.50–15.30) consistent with penetrating artery disease as the most likely etiology. DWI based studies exploring differences in stroke mechanisms and etiology between the vascular territories are predominantly based on the TOAST classification system of ischemic stroke subtypes, and have often been conducted in populations of east Asian decent[45, 58] which limits reliable comparison with our study population which is predominantly of European descent.

To conclude, clinical use of MRI-DWI to confirm PCiS improves accuracy of diagnosis, and classification of etiology, and contributes to the reliability of risk factor data and will help validate causal links regarding underlying stroke risk factors related to subtype.

Genetic overlap between migraine and PCiS vs. ACiS (III)

In this study we used well-defined MRI–DWI phenotypes of acute PCiS and ACiS to investigate differences in genetic contribution to the risk of PCiS vs. ACiS. We constructed polygenic risk scores for three migraine phenotypes and compared their association between PCiS and ACiS, and between each stroke subtype and non-stroke control subjects.

We found a shared genetic contribution to migraine (M) and migraine without aura (MO) and PCiS. In contrast, the results do not indicate a shared genetic background between migraine and ACiS. When we compared migraine PRSs in PCIS and ACiS separately against published results from a large group of non-stroke control subjects available through SiGN,[80] the association of M PRSs and MO PRSs remained stronger

for PCiS than for ACiS. Compared to non-stroke control subjects M PRSs were associated with an increased risk of PCiS while the risk for ACiS was decreased.

In our study, PRSs for migraine *with* aura (MA) did not show any association for either stroke phenotype. This is in contrast to epidemiological data indicating that this migraine phenotype in particular is most strongly associated with the risk of ischemic stroke.[146-148, 196]

Since PCiS has not previously been investigated specifically and separately from ACiS in any ischemic stroke GWAS or other studies of genetic overlap with migraine, it is difficult to make direct comparisons of our results with any previous reports. When shared genetic contribution between migraine and ischemic stroke, and ischemic stroke subtypes, was explored in a recent genetic association study[126] using four methods of genetic analyses, MO derived PRSs showed significant associations with all IS, especially with the subtypes large artery stroke and cardioembolic stroke. MA derived PRSs showed much weaker association to IS overall and to larger artery stroke. The genetic overlap and correlation between MA and ischemic stroke was weaker than for MO overall. The IS subtype SVD showed the weakest genetic overlap and genetic correlation with all migraine phenotypes in the same study.

Because of the limited sample size of PCiS and ACiS we did not investigate migraine PRSs in relation to ischemic stroke subtypes. However, the IS subtype distribution differed significantly between the vascular territories in our IS genetic cohort: SVD was 23% in the PCiS group vs. 16% in ACiS, and LAA was 21% PCiS and 33% in ACiS. GWAS of ischemic stroke subtypes have identified fewer susceptibility loci for SVD than for other stroke subtypes and the genetic association study of migraine and IS mentioned above showed the weakest association between migraine phenotypes and SVD compared to CE and LAS. In contrast, despite a smaller population of PCiS than ACiS and a higher proportion of SVD in the PCiS group we detected a consistently stronger genetic overlap between migraine and PCiS than ACiS, including for non-stroke control subjects. It may be that the subtyping of ischemic stroke according to vascular territory also enriched aspects of the SVD subtype and that shared genetic contribution to migraine and PCiS is linked to mechanisms related to small-vessel function, but this is speculative.

The lack of any significant or differential association between MA derived PRSs and either PCiS or ACiS in our study could be related to several factors. Migraine GWA studies have so far not identified any MA associated SNPs of genome-wide significance ($\leq 1E^{-8}$), and a fewer number of associated SNPs above the significance level ($>1E^{-8}$) than for M and MO, which led to fewer MA related SNPs being included in our study. In addition, the weaker findings for MA than for M or MO associated SNPs in large

migraine GWA studies may reflect a relatively larger contribution of rare rather than common genetic variants to MA.[125]

Our target cohort of ischemic stroke patients was small which prevented investigations on the influence of sex on the results. Migraine prevalence is higher in women than in men and migraine incidence typically peaks in mid-life,[145] whereas ischemic stroke generally occurs in older populations. In paper II, we also showed that male sex was more strongly associated with PCiS, in line with other studies with well-defined PCiS populations.[7, 65, 189] It may indicate that genetic contribution is either enhanced or mitigated by the influence of other factors such as hormonal status.

Differences between the posterior and anterior circulation in cerebral vascular reactivity as a marker of cerebral vessel endothelial function have been demonstrated in MA patients only in one study,[197] and in both MA and MO patients in another study.[198] The role of systemic endothelial dysfunction is less clear.[36, 199, 200] Endothelial cell signalling pathways and vascular smooth muscle function have been implicated in migraine pathophysiology. The largest GWAS of migraine to date identified several loci linked to genes associated with vascular and smooth muscle function.[127] Though involvement of the posterior circulation in infarct–like lesions and migrainous stroke primarily has been shown for migraine with aura,[164] it may suggest endotheliopathy or variation of the endothelium in the posterior circulation compared with the anterior circulation as the common link in vascular brain changes in the posterior circulation territory and migraine. A recent genetic fine mapping study implicated endothelin 1 in several vascular diseases including migraine.[201] Endothelin 1 is a peptide with potent vasoconstrictive actions that may be involved in migraine.[202] Endothelial dysfunction implicates many associated mechanisms such as regulation of vascular tone, thrombosis and inflammatory response, all of which has a role in ischemic stroke pathogenesis. Seen in context of our finding in paper II of an association between diabetes mellitus and PCiS, endothelial injury and dysfunction may play a different and more prominent role in PCiS than in ACiS.

One strength of the current approach is that the genetically based PRS method is less sensitive to the influence of confounding factors, such as life-style choices and medication use, than epidemiological methods, though genetic influence may be mediated by environmental exposure. Even if the increase in risk was modest the strength of our results is in the consistency of association with PCiS rather than ACiS across analyses. To confirm the association, our results need to be replicated in a larger, independent populations of well-defined PCiS and ACiS. The results do not establish a common causative mechanism in migraine and PCiS, but may point to a common underlying dysfunction predisposing for both conditions.

Our findings indicate a difference in genetic background regarding the risk of PCiS vs. ACiS. A genetic risk score for ischemic stroke incorporating multiple distinct GRSs for stroke and known stroke risk factors has recently been reported to identify individuals with increased genetic risk of ischemic stroke.[139] Genetic risk scores for stroke will likely play an increasing role in risk stratification, personalized and preventive medicine. Integrating a migraine GRS into future risk prediction models for ischemic stroke, or investigating if existing metaGRSs have a differential impact on the risk of PCiS vs. ACiS may identify specific PCiS risk factors and lead to more targeted PCiS management.

Prevalence and laterality of FTP in two patient populations (IV)

We investigated the prevalence and laterality of partial and complete fetal posterior cerebral arteries (FTP) on MRA in ischemic stroke patients with confirmed DWI lesions. We compared our findings with an unselected hospital–based patient population investigated with CTA.

There are few MRA based studies on ischemic stroke populations in which FTP prevalence and laterality have been systematically evaluated. MRA studies in healthy populations report FTP prevalence of 13%–32% and differ in their study sizes and definitions of FTP.[24, 203-205]

We found any type of FTP in approximately 30% of patients in both the AIS population investigated with MRA and in the unselected hospital–based population investigated with CTA. Outside of autopsy case series, reports on the prevalence of a fetal posterior cerebral artery in AIS are few. A mixed CTA/MRA based study in AIS patients found FTP in 25% of patients.[179] Some have primarily focused on investigating the relationship between AIS or lesion location and the presence of FTP in select groups, such as only patients with ICA stenosis or with PCA infarctions.[86, 206]

The laterality of FTP has not been paid much attention before. We found right–sided FTP to be near twice as frequent as left–sided FTP in both the AIS group and the unselected hospital–based group. A similar discrepancy has been reported in other studies reporting laterality but was not discussed further, perhaps because of limited sample sizes.[24, 179, 207] Our finding is interesting in relation to hypoplasia of the vertebral artery which also has been observed more frequently on the right side in both AIS patients and healthy populations.[208-210] Vertebral artery hypoplasia was associated with the presence of FTP in a large study on healthy subjects, and the proportion of patients with FTP increased with the degree of vertebral artery hypoplasia showing the strongest association with the vertebral artery ending in a posterior cerebellar artery.[24] The same study found FTP to be ipsilateral to the hypoplastic vertebral artery more frequently.

A possible (but speculative) explanation for the preferential lateralization of FTP to the right could be that the presence of a right hypoplastic vertebral artery during fetal development inhibits the regression of the ipsilateral persisting fetal posterior artery to the normal adult configuration, in order to ensure perfusion to the posterior brain. We did not evaluate the vertebral artery for presence of hypoplasia in the AIS population and could not confirm its association with FTP.

Bilateral FTP was more common in women than in men in both of our study populations. Studies generally do not report sex specific prevalence data for FTP, but one study found that complete FTP was more common among women.[179] A biological explanation for these differences is difficult to find in the literature. However, exclusive dependence on the carotid system for perfusion of the entire supratentorial brain increases vulnerability to ischemic injury and stroke, especially in patients with bilateral complete FTP who have no collateral reserve via the posterior circulation.

We analysed if PCA territory involvement on MRI–DWI occurred more often in patients with FTP on the lesion side than in patients with no FTP on the lesion side and found no evidence for that. This is in keeping with one recent study, similar to ours in method and objective, in which FTP did not influence stroke side or vascular territory involved.[179] In our study prevalence of FTP in the AIS group was similar to that reported in healthy populations and to our hospital–based unselected population, making it unlikely that FTP alone increases the risk of ischemic stroke. No prospective cohort studies designed to establish if the risk of ischemic stroke differs between individuals with and without FTP independently of other vascular risk factors have been published.

We found FTP in close to a third of ischemic stroke patients which has implications in clinical practice. Patients with FTP ipsilateral to stroke lesion in conventional PCA territory and high grade stenosis of the ipsilateral ICA should be evaluated for carotid endarterectomy as a possible secondary prevention measure[206, 211] They are also at risk for extensive hemispheric ischemic injury encompassing both MCA and PCA territories.[212, 213] The influence of FTP on imaging results, i.e. perfusion patterns in acute ischemia has not been studied extensively, but may lead to left–right asymmetry in the posterior territory, mimicking cerebrovascular compromise.[214, 215]

This study primarily highlights the high prevalence of FTP variants in ischemic stroke patients, with the additional findings of a laterality preference to the right side, possibly as a consequence of interdependence between the anterior and posterior arteries during fetal development. As mentioned, we were unable to investigate FTP in relation to vertebral artery hypoplasia in ischemic stroke patients and cannot be sure if the detection of one variant should lead to high suspicion of the other.

Methodological considerations

Each SiGN site contributing MRI data to MRI-GENIE collected data on acute stroke patients according to a predefined protocol common to all participating sites. Some of the sites incorporated the protocol into pre-existing stroke registers and local studies. Consequently, one of the important systematic errors at work in this thesis is selection bias derived from the included sites.

Selection bias

Selection bias occurs when participants in a study differ from the population of interest in a systematic way and therefore are not representative of the population the study is intended to investigate. One result of selection bias can be skewed measures of associations that are not valid for the target population. Clinical trials utilize randomization of patient groups as one strategy to minimize selection bias.

In cross-sectional studies, patients (study subjects) are chosen from an available population based on the relevance the population may have to a research question. Two different categories of selection bias are sampling bias and prevalence/incidence bias. Sampling bias occurs when some individuals in a population of interest (ischemic stroke) are more likely to be included than others, and prevalence/incidence bias when an individual's disease status (mild or severe) leads to exclusion.

For this thesis the MRI-GENIE population was chosen because we were interested in a genotyped acute ischemic stroke population with MRI–DWI data enabling diagnosis of posterior and anterior circulation ischemic stroke to investigate vascular risk factor differences and genetic overlap in these stroke types. As an example of sampling bias, patients at participating SiGN/MRI-GENIE sites are included only if they can actively consent to donating biosamples for genotyping. There is a possibility that patients with severe stroke, or aphasia, were unable to consent, in which case this study sample may be more representative of milder strokes.

Our study requirement of available MRI scans introduces selection bias for several reasons. MRI imaging was not a prerequisite inclusion criterion in the original SiGN protocol. Some sites routinely used MRI as part of acute stroke imaging in clinical practice while others performed MRI only on clinical indication. The proportion of AIS patients receiving MRI imaging among the SiGN/MRI–GENIE sites reporting data on MRI use in the cohort is between 20%–100%.[119] The use of MRI-imaging in the acute phase or as part of inpatient work up after CT is institution dependent and limited by contraindications, lack of availability and patient clinical factors. Older and

medically unstable patients, and patients with severe deficits are more likely to not undergo MRI.[216, 217] This type of selection bias may have influenced the composition of the cohort. It may partially explain the relatively low mean age in MRI–GENIE (62.4 years) which in turn may have influenced the prevalence of vascular risk factors which increases with age.

Between the years 1999–2008 the use of brain MRI in stroke increased 38% as studied in 11 US states and the relative increase in proportion of stroke patients imaged with MRI was 235%.[218] This period roughly overlaps with the inclusion period for the SiGN/MRI–GENIE sites.

Additional bias may have been introduced by including only patients with visible DWI lesions on MRI in our analyses in papers II–IV. This meant excluding >300 patients with clinical stroke and available MRI-DWI scans but no visible DWI abnormality in the MRI-GENIE study. Negative DWI on acute imaging occurs in a proportion of AIS patients and has been associated with hyperacute imaging (<3 hours),[105] lacunar stroke, and posterior circulation location, particularly brainstem location.[219] Consequently, some of the patients in whom we detected no DWI lesions may have been PCiS patients who were not included in our analyses in studies II–IV.

On the other hand, MRI is often used as a complement instead of a replacement for, CT[218] and unremarkable CT scans in patients with strong clinical suspicion of stroke may have been selected for complementary MRI investigations. Given the lower sensitivity for ischemic injury in the posterior circulation on CT[51] a disproportionate number of PCiS patients may have had MRI as part of their inpatient workup leading to overrepresentation in our study.

Cross-sectional design

Cross-sectional studies are particularly suited for estimating the prevalence of a disease or a trait in the general population or in a specific patient population (Paper IV).[220] They measure and record variables of interest at a single time point, and do not include repeated measurements of exposures and outcomes longitudinally in a study population. For that reason, they cannot be used to infer causal effects or relationships between a known risk factor and a disease. However, associations between an exposure and outcome can be calculated from data in a cross-sectional sample and may be hypothesis generating (Paper II) especially when many other cross-sectional studies with comparable data show similar associations. Cross-sectional samples are also suitable for studying factors that are expected to be stable over time and unlikely to be significantly influenced by environmental or life-style factors. Examples of exposures

that preceded the studied outcome are congenital anatomical structures (Paper IV) or genetic markers (Paper III).

Ethnic diversity in genetic association studies

An overwhelming proportion of GWA studies (80% in 2019) has been conducted in individuals of European ancestry.[221] Studies on other populations have often been of limited sample size.[222] Most of the genetic variation present in the world population has thus been insufficiently examined and not incluced into current knowledge of the genetic architecture of complex diseases. The lack of diversity can lead to genetic variants of importance being missed because they are absent or occur only at low frequency in European populations.[223] Hypotheses concerning biological mechanisms of disease and risk predictions derived from GWA studies on single ancestry populations cannot be directly applied in others.[224]

In our study (III) of genetic overlap between migraine and posterior vs. anterior circulation ischemic stroke, polygenic risk scores were developed using summary results from a meta-analysis of 22 previously conducted migraine GWA studies.[127] It included 59 674 cases and 316 078 control subjects, all of whom were of European ancestry. Our primary analyses therefore only included ischemic stroke cases of European ancestry. PRSs constructed from GWA studies on European samples perform poorly in other ancestries, and this is especially true for African populations.[225] Our primary analyses only included ischemic stroke cases of European ancestry. The stronger association of migraine PRSs with PCiS compared with ACiS can consequently not be directly extrapolated to population of different ethnicities, which limits the generalizability of our results.

Other methodological considerations in GWA studies and PRS

In statistical hypothesis testing a type I error means finding a statistically significant association when in fact there is no true association (false positive). The conventionally used significance level of $p<0.05$ means that 5% of the time we would find a false positive and reject the null hypothesis of no association despite the fact that it is true. This is true for a single statistical test and thus has specific consequences in GWA studies in which millions of SNPs are compared and each test brings its own risk of introducing a Type I error. The standard method of correcting for multiple testing in GWA studies is the Bonferroni method in which the significance level of 0.05 is corrected to 0.05/number of statistical tests performed. If using 500 000 SNPs, the significance of a SNP association would be set at 1e-7. The standard for significance in

GWAS on European ancestry samples is set to $p<5\times10^{-8}$. This stringent correction is one of the reasons that very large sample sizes are required, and it may increase the number of Type II errors, i.e. failure to find an association when indeed there is one (false negative).

Originally, PRS studies incorporated only gene variants that were below the threshold for significance in the discovery GWAS, but including variants that are above the threshold has proven useful in increasing the predictive power of PRSs. We included SNPs at 4 different significance levels, 3 of which are above the standard p-value threshold of 10^{-8} for GWAS significance. We used PRSs as a method to explore shared etiology between migraine and PCiS. The association of a PRS with different phenotypes may be due to biological mechanisms with separate and direct effects (horizontal pleiotropy) on the investigated phenotypes, or to different downstream effects in a molecular pathway (vertical pleiotropy).[133] The pleiotropic effect may mean that a polygenic risk score developed in one trait associates with one or several other traits, meaning that the common genetic variants that constitute the score influence the risk of one or more other diseases. We did not evaluate the specificity of the migraine PRS, i.e. it was not tested against a range of other traits associated with ischemic stroke such as diabetes or cardiovascular disease. The occurrence of gene variants with effect on different traits is also abundant in the human genome and the genetic sharing is not evenly distributed between traits. It is therefore not certain that a SNP with a high effect size in the discovery GWAS of one trait has any effect in the target population.

Manual data entry

Manual data entry by humans will inevitably result in error with the potential to generate inaccurate statistical analyses results, leading to incorrect research conclusions. The most common error in this procedure is a typo, i.e. 3 becomes 2, or 33, which may lead to the incorporation of values with effects on correlations, associations or significance.[226] Error probability has been estimated between 18–40%.[227]

The structured protocol developed for Paper I and used for analyses in the subsequent studies II–IV exemplifies manual data entry. In total it contains 74 variables pertaining to every study individual from the 12 sites. Input alternatives for most variables were single digits between 0 and 3. The neuroradiologist visually assessed the neuroimaging data for each variable in the data sheet (e.g. left brainstem lesion, Pcom>P1) and entered a number in the corresponding column. Each study site had a separate spread sheet. After data entry by the neuroradiologist the site sample was sent for data checking by another individual. This included visual checking of all data entries for errors that produced implausible results. For instance, to be assigned 1 (=Yes) in the column for

FPCA (fetal type PCA), the combination of digits in the previous columns for Pcom and P1 had to be 2–1 or 2–0, otherwise the entry for FPCA should be 0.

All variables for each study individual were checked in this manner and by cross tabulation in SPSS. Data sheets were returned to the neuroradiologist with requests to recheck imaging data when necessary. Vascular territory had to be reassigned after initial data entry in less than <1% of cases.

Conclusions

- Multicenter collaborations to accrue MRI data of hospital-based stroke populations allow integration of neuroimaging features into the stratification of IS patients resulting in more homogenous subgroups and enriched stroke phenotypes while preserving sufficient sample sizes for research.
- Vascular risk factors differ in prevalence and strength of association between PCiS and ACiS. Diabetes mellitus and male sex are more strongly associated with PCiS than with ACiS.
- Stroke etiologies contribute in different proportions to PCiS vs. ACiS, and DWI lesion distribution patterns in PCiS may aid in determining etiology.
- Careful delineation of PCiS and ACiS based on MRI imaging in combination with clinical data and genetic association analyses may elucidate genetic factors contributing to the risk of PCiS. Understanding genetic factors and biological mechanisms could inform strategies for treatment and prevention.
- Certain migraine phenotypes share a proportion of their genetic architecture with PCiS among individuals of European ancestry, and polygenic risk scores derived from these migraine phenotypes increased the risk of posterior rather than anterior circulation ischemic stroke.
- FTP is present in up to one third of ischemic stroke patients and unselected hospital-based patients alike, which indicates that FTP is unlikely to increase the risk of ischemic stroke in the absence of other risk factors. There is a preferential lateralization to the right of FTP, but an association between the side preference and other lateralized variants in the vertebrobasilar circulation need further study.

Populärvetenskaplig sammanfattning

Stroke

Stroke är ett samlingsbegrepp för flera tillstånd som drabbar hjärnan och centrala nervsystemet. Stroke indelas brett i ischemisk stroke och hjärnblödning. Ischemisk stroke uppstår när ett blodkärl till eller i hjärnan eller ryggmärgen täpps till, vanligtvis av blodpropp, vilket orsakar syrebrist och vävnadsdöd. Hjärnblödningar delas in i intracerebrala (i hjärnan) eller subarachnoidala (under hjärnhinnan). Ischemisk stroke utgör ca 80–85% av alla stroke, medan intracerebrala och subarachnoidala blödningar utgör respektive ca 15% och 5%. Stroke är en global folksjukdom och en ledande orsak till död och varaktig funktionsnedsättning i världen. Det finns många möjliga orsaker och underliggande mekanismer till ischemisk stroke. Generella mekanismer är blodproppar som antingen bildas lokalt i ett hjärnkärl (trombos) eller når hjärnan via blodflödet, t.ex. från hjärtat (embolism). Underliggande processer kan vara påverkan på hjärnans små kärl, åderförkalkning eller proppbildning i hjärtat på grund av rytmrubbning eller annan hjärtsjukdom. Kända riskfaktorer för ischemisk stroke är högt blodtryck, förmaksflimmer, diabetes och höga kolesterolvärden.

Avhandlingens fokus och målsättning

Den här avhandlingen handlar om ischemisk stroke som drabbar hjärnans bakre kärlområde (häri används förkortningen PCiS). I det området ingår hjärnstammen, lillhjärnan och de bakre delarna av storhjärnan samt andra djupt liggande strukturer i storhjärnan. Dessa strukturer försörjs med syrerikt blod av artärer som tillsammans utgör vertebrobasilaris-systemet (VB). I avhandlingens introduktion finns en illustration över dessa artärer. Orsaker till ischemisk stroke i det här området är generellt desamma som i det främre området, eller karotis-området. Karotiderna är de stora halspulsådrorna.

Ofta förknippas stroke-symptom med plötsliga svårigheter att tala eller förstå, förlamning eller halvsidig svaghet i extremiteterna, och känselbortfall. Eftersom hjärnstrukturerna som försörjs av det bakre kärlsystemet har så varierande funktioner kan stroke i detta område ge helt andra symptom, ibland i symptomkonstellationer som är svårtolkade. Yrsel, synfältsbortfall, artikulationssvårigheter, illamående, sidoväxlande

svaghet, balans- och koordinationssvårigheter är vanliga symptom på stroke i det bakre kärlområdet. Hjärnstammen reglerar livsviktiga funktioner som andning och hjärtrytm och allvarliga stroke i detta område kan leda till döden eller allvarlig funktionsnedsättning oavsett behandling. Symptomen kan också vara så lindriga och snabbt övergående att den drabbade inte söker sjukvård eller att diagnosen missas trots att hen har sökt sjukhusvård. Forskning om och behandling av PCiS har i förhållande till stroke i det främre kärlområdet historiskt varit eftersatt. Det beror bland annat på att kärlanatomin skiljer sig åt mellan främre och bakre området, att diagnosen PCiS kan vara svår att ställa baserat på bara symptombild, och att PCiS har exkluderats, varit underrepresenterat eller inte specifikt utvärderats i stora kliniska behandlingsstudier. Det finns även skillnader mellan de två kärlområdena avseende kärlens utveckling under fosterstadiet och i kärlväggens uppbyggnad och sammansättning. Mycket stora framsteg inom urakut behandling av ”karotis-stroke” har gjorts de senaste 5–10 åren och sedan tidigare har ingrepp för att förebygga nya stroke kunnat erbjudas patienter med karotis-stroke om orsaken är förkalkningar i halspulsådrorna. Samma typ av behandling och förebyggande åtgärder kan inte erbjudas patienter med PCiS i dagsläget och därför är det viktigt att forskning som särskilt fokuserar på PCiS bedrivs.

En förutsättning för att kunna studera ett sjukdomstillstånd är att de individer som studeras har rätt diagnos. Utvecklingen av allt bättre bildframställningsmetoder som magnetresonanstomografi (MRT eller MRI) har gjort att PCiS-diagnos idag kan ställas med större säkerhet än tidigare. Andra röntgenmetoder är sämre på att avbilda strokeskador i det bakre kärlområdet, särskilt i hjärnstammen.

Delarbeten

Avhandlingen studerar bakre kärlområdet och PCiS ur flera aspekter. Den är baserad på ett stort antal MR-undersökningar av akuta strokepatienter, insamlade vid 12 olika center i USA och Europa. Alla individer som ingår i studien har en säkerställd diagnos på ischemisk stroke i antingen bakre eller främre kärlområdet. Vi hade också tillgång till information om strokeriskfaktorer, strokeorsak och genetisk sammansättning, så kallad genotyp-data, för alla individer.

(I) I detta arbete granskade vi 3 301 MR-undersökningar av akuta strokepatienter för att identifiera vilka som var PCiS och ACiS, noga kartlägga strokeskadornas utseende och utvärdera hjärnkärlen avseende anatomi och åderförkalkningsgrad.

(II) Förekomst av kända strokeriskfaktorer som högt blodtryck, diabetes och förmaksflimmer analyserades och jämfördes mellan PCiS och ACiS. PCiS-gruppen

studerades också avseende skadornas lokalisation i hjärnstammen, lillhjärnan eller storhjärnan.

(III) Vi ville undersöka om det finns genetiska faktorer som ökar risken för eller har ett starkare samband med PCiS jämfört med stroke i främre kärlområdet. I andra typer av studier har möjliga samband mellan PCiS och migrän visats, vilket vi utnyttjade som modell in denna genetiska studie. Vi konstruerade en ”genetisk profil” eller genetisk riskskala (polygenic risk score) som avspeglar risken för migrän och tillämpade denna på strokegrupperna och på en stor grupp kontrollindivider utan ischemisk stroke.

(IV) Förekomst och sidolokalisation av en anatomisk variant i ett av de kärl som försörjer hjärnans bakre delar studerades i två olika patientgrupper. Vi analyserade också om risken för att en strokeskada ska drabba just det bakre kärlområdet är större hos individer med den studerade kärlvarianten än i individer utan denna variant.

Resultat och konklusioner

Sammanfattningsvis visade vi att närmare en tredjedel av de 3 301 strokepatienterna hade PCiS. I litteraturen uppges ofta andelen PCiS vara 20–25 %. I den jämförande analysen av strokeriskfaktorer visade vi att diabetes mellitus och manligt kön har en starkare koppling till PCiS än till ACiS. Samma resultat har visats i tidigare studier, men resultaten är inte helt enhetliga mellan studier, vilket kan bero på att PCiS-diagnosen har ställts på olika sätt i olika studier. Det går inte att uttala sig angående direkta orsakssamband eftersom vår studie är en tvärsnittsstudie. Vi visade också att den genetiska variation som bidrar till risken för migrän också bidrar till risken för PCiS i större utsträckning än till ACiS. Fyndet behöver bekräftas i större studier, men pekar på att det kan finnas mekanismer på molekylär nivå som skiljer sig mellan de två stroketyperna. I delarbete IV påvisades den medfödda kärlvariant som benämns *fetal-type posterior cerebral artery* (FTP) lika ofta i vår studiepopulation med stroke som i en jämförelsegrupp utan strokediagnos som undersökts med kärlröntgen av olika anledningar. Varianten förekom hos 30 % i båda grupperna, vilket talar emot att den i sig ökar risken för ischemisk stroke. I både grupperna förekom FTP avsevärt oftare på höger sida i hjärnan än vänster och skillnaden är statistiskt säkerställd. FTP har praktisk relevans i utredningen och behandlingen av ischemisk stroke eftersom de delar av storhjärnan som annars försörjs av bakre cirkulationen, istället försörjs via FTP och karotiskärlen. Det betyder att en strokeskada i hjärnans bakre delar kan uppstå på grund av en blodpropp som tagit sig dit via halspulsådern på samma sida.

Sammanfattningsvis påvisar huvudfynden i avhandlingen att det finns viktiga skillnader mellan PCiS och ACiS och att dessa skillnader delvis kan vara genetiskt betingade.

Diabetes som är en riskfaktor för all stroke visade sig ha en starkare koppling till PCiS än ACiS. Det kan betyda att kärlväggarna i det bakre kärlområdet är mer sårbara för diabetesskador än kärlen i det främre området. Denna koppling bör beforskas vidare med olika metoder. Studier som förenar pålitlig diagnostik av PCiS med genetik är en lovande metod för att identifiera specifika sjukdomsmekanismer eller riskfaktorer som bidrar till PCiS. I förlängningen skulle det kunna leda till nya behandlingsmöjligheter eller förebyggande åtgärder för individer med risk för PCiS.

Future perspectives

Medical research is motivated by the aspiration to improve patient treatment and prevent disease. Improvement in diagnostic accuracy, treatment and prevention measures in PCiS are needed for several reasons. PCiS is missed both clinically and by neuroimaging to a greater extent than anterior circulation stroke. This may mean missed opportunities for secondary prevention measures and an increased risk for stroke recurrence in affected patients. Recurrence rates in vertebrobasilar TIAs and minor stroke are at least as high as in the carotid artery system, and secondary prevention measures are in a majority of cases limited to pharmacological treatment, while carotid endarterectomy to prevent recurrent stroke in ACiS caused by significant stenosis of the internal carotid arteries is routine. Identifying specific mechanisms of pathogenesis and injury relevant in posterior circulation arteries as targets for primary or secondary prevention measures may improve prognosis in these patients.

To that end, some of our findings in this thesis help identify areas for future research. There are several studies showing a stronger association between diabetes mellitus and PCiS vs. ACiS, in line with our findings in the MRI-GENIE population. A Mendelian randomization study, recently indicated that type 2 diabetes mellitus may play a causative role in the ischemic stroke subtype LAA, but not in small vessel or cardioembolic stroke.[228] Exploring a genetic basis for the association between T2D and PCiS by using well defined populations of PCiS and ACiS stratified by ischemic stroke subtypes in a similar study design may reveal if T2D has specific significance in PCiS. For instance, establishing diabetes mellitus as a particular risk factor in PCiS would be of importance in determining if levels of glycemic control should be more stringent as a secondary prevention measure in this patient group. Similar studies may also help to elucidate any specific mechanisms of atherogenesis in posterior circulation vessels. Differences in the arterial walls in response to ageing has been shown for intracranial vessels in the anterior and posterior circulation. Age related histopathological differences between the vascular beds, such as greater elastin loss, intimal thickening and increased proportion of internal elastic lamina in the posterior arteries have been reported.[229]

Our study on genetic overlap between migraine phenotypes and PCiS indicated a shared genetic background of common variants that was not evident for ACiS. The study needs to be replicated with larger, independent samples of PCiS and ACiS patients to confirm our findings, and the specificity of the PRS for migraine needs to be confirmed by applying it to several other traits. However, the results are hypotheses generating in terms of shared biological mechanisms, perhaps converging on endothelial function. Further insights into underlying mechanisms and biological pathways relevant to PCiS specifically could be gained by stratifying our genetic association analyses according to ischemic stroke subtypes. For instance, in the present PRS study the proportion of SVD was much higher in the PCiS than in the ACiS group, which raises questions about whether the PRS association was driven by the additive effect of common variants with implications in small artery dysfunction, relevant to prevention of both migraine and stroke. A replication study of sufficient sample size would help answering or narrowing that question.

The findings in this thesis rest entirely on an accurate diagnosis of PCiS achieved by MRI-DWI imaging. A definitive diagnosis of PCiS will be essential in future studies aiming to identify specific risk factors or underlying mechanisms for this stroke subtype. Stroke research is increasingly incorporating imaging characteristics to achieve enriched stroke phenotypes. The Swedish national hospital-based stroke register Riksstroke has a coverage rate of 89% (2019) for stroke in Swedish hospitals. It is primarily a quality register for stroke care but is widely used for clinical and epidemiological stroke research. It does not include designation of vascular territory of stroke as a diagnostic variable. Imaging finding of included patients are not registered and source images are not available since data is anonymized. Including information on vascular territory in Riksstroke or similar hospital-based registers, combined with radiology reports or image data would facilitate future research on PCiS.

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Appendix

Table 1.
Study Protocol for evaluation of MRI/DWI and MRA studies in the MRI-GENIE ischemic stroke cohort

1	StudyID	Study Center
2	ID	Numeric
3	Gender	M and F
4	Race	
5	Ethnicity	
6	Age At Stroke	
7	Infarct Location CENTER	
8	Time To Scan	Numeric
9	MRA Available	0=No, 1=Yes
10	Infarct Location DWI_JW	
11	Vascular Territory	0=Undecided, 1=Anterior, 2=Posterior, 3=Both
12	Vascular Side	1=Left, 2=Right, 3=Both
13	Left Brainstem	0=No, 1=Yes
14	Right Brainstem	0=No, 1=Yes
15	Right Brainstem	0=No, 1=Yes
16	Right Brainstem	0=No, 1=Yes
17	Right Brainstem	0=No, 1=Yes
18	Multiple Brainstem	0=No, 1=Yes
19	Left/Right Cerebellum	0=No, 1=Yes
20	Multiple Left/Right Cerebellum	0=No, 1=Yes
21	Left/Right Posterior Cortical	0=No, 1=Yes
22	Left/Right Posterior Deep	0=No, 1=Yes
23	Multiple Left/Right Posterior	0=No, 1=Yes
24	Lacunar Left/Right Posterior	0=No, 1=Yes
25	Left/Right MCA Cortical	0=No, 1=Yes
26	Left/Right MCA Deep	0=No, 1=Yes
27	Multiple Left/Right MCA	0=No, 1=Yes
28	Lacunar Left/Right MCA	0=Open, 2= Stenosis, 3= Occlusion
29	Left/Right ACA Cortical	0=Open, 2= Stenosis, 3= Occlusion
30	Left/Right ACA Deep	0=Not Visible, 1=< P1, 2= > P1
31	Basilar Artery	0=No, 1=Yes
32	Vertebral Left/Right	0=Open, 2= Stenosis, 3= Occlusion
33	Pcom Left/Right	0=Open, 1=Not visible
34	Fetal Type PCA Left/Right	0=Open, 2= Stenosis, 3= Occlusion
35	PCA Left/Right Occlusion	0=Open, 2= Stenosis, 3= Occlusion
36	Acom	0=Open, 2= Stenosis, 3= Occlusion
37	C5 Left/Right	0=Open, 2= Stenosis, 3= Occlusion
38	ACA Left/Right Occlusion	0=No, 1=Yes
39	MCA Left/Right Occlusion	
40	ICA Left/Right Occlusion	0=No, 1=Yes
41	MRA Occlusion Related to Location Of Acute Stroke	0=No, 1=Yes
42	Specify Location MRA Occlusion	1=Pcom R, 2=Pcom L, 3=Acom, 4=Other
43	Other Finding	
44	Specify Other Finding	0=No, 1=Yes
45	Aneurysm	
46	Location Aneurysm	0=No, 1=Yes
47	Specify Other Location Aneurysm	1=ICAR, 2=ICAL, 3=VaR, 4=VaL
48	Ectasia	0=No, 1=Yes
49	Specify Location Ectasia	
50	Dissection	
51	Location Dissection	Study Center
52	Other Location Dissection	Numeric
53	Specify Other Location Dissection	M and F
54	COMMENTS	
55	Right Brainstem	
56	Multiple Brainstem	
57	Left/Right Cerebellum	
58	Multiple Left/Right Cerebellum	Numeric
59	Left/Right Posterior Cortical	0=No, 1=Yes
60	Left/Right Posterior Deep	
61	Multiple Left/Right Posterior	0=Undecided, 1=Anterior, 2=Posterior, 3=Both

62	Lacunar Left/Right Posterior	1=Left, 2=Right, 3=Both
63	Left/Right MCA Cortical	0=No, 1=Yes
64	Left/Right MCA Deep	0=No, 1=Yes
65	Multiple Left/Right MCA	0=No, 1=Yes
66	Lacunar Left/Right MCA	0=No, 1=Yes
67	Left/Right ACA Cortical	0=No, 1=Yes
68	Left/Right ACA Deep	0=No, 1=Yes
69	Basilar Artery	0=No, 1=Yes
70	Vertebral Left/Right	0=No, 1=Yes
71	Pcom Left/Right	0=No, 1=Yes
72	Fetal Type PCA Left/Right	0=No, 1=Yes
73	PCA Left/Right Occlusion	0=No, 1=Yes

DWI = Diffusion-weighted imaging; MCA, middle cerebral artery; ACA, anterior cerebral artery; Pcom, posterior communicating artery; PCA, posterior cerebral artery; Acom, anterior communicating artery; ICA, internal carotid artery; MRA, magentic resonance angiography

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