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Brain plasticity and network reorganization in children with perinatal stroke: a functional magnetic resonance imaging study

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1 LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications that are referred to in the text by their Roman numerals.

I. Ilves, Nigul; Lõo, Silva; Ilves, Norman; Laugesaar, Rael; Loorits, Dagmar; Kool, Pille; Talvik, Tiina; Ilves, Pilvi (2022). Ipsilesional volume loss of basal ganglia and thalamus is associated with poor hand function after ischemic perinatal stroke. BMC Neurol. 22, 23. https://doi.org/10.1186/s12883-022-02550-3

II. Ilves, Nigul; Ilves, Pilvi; Laugesaar, Rael; Juurmaa, Julius; Männamaa, Mairi; Lõo, Silva; Loorits, Dagmar; Tomberg, Tiiu; Kolk, Anneli; Talvik, Inga; Talvik, Tiina (2016). Resting-State Functional Connectivity and Cognitive Impairment in Children with Perinatal Stroke. Neural Plasticity, 2016, 11−11. DOI: 10.1155/2016/2306406.

III. Ilves, Nigul; Männamaa, Mairi; Laugesaar, Rael; Ilves, Norman; Loorits, Dagmar; Vaher, Ulvi; Kool, Pille; Ilves, Pilvi (2022). Language lateralization and outcome in perinatal stroke patients with different vascular types. Brain and Language 228, 105108. https://doi.org/10.1016/j.bandl.2022.105108

Applicant's contributions to papers I–III: Nigul Ilves was involved in the design of the study, data collection (partially), data analysis and statistics, writing the manuscripts and publishing.

2 ABBREVIATIONS

AHA	Assisting Hand Assessment
AIS	Arterial Ischemic Stroke
AT	Anterior Trunk
CI	Confidence Interval
DMI	Distal M1 Infarction
EV	Expressive Vocabulary Test
FCI	Fluid-Crystallized Index
FDR	False Discovery Rate
fMRI	Functional Magnetic Resonance Imaging
FSL	FMRIB (Functional Magnetic Resonance Imaging of the Brain) Software Library
LI	Lateralization Index
MACS	Manual Ability Classification System
MCA	Middle Cerebral Artery
MPI	Mental-Processing Index
MRI	Magnetic Resonance Imaging
NA	Not Available
NVI	Nonverbal Index
PMI	Proximal M1 Infarction
PSOM	Pediatric Stroke Outcome Measure
PT	Posterior Trunk
PVI	Periventricular Venous Infarction
RID	Riddles Test
ROI	Region Of Interest
SCT	Sentence Comprehension Task
SD	Standard Deviation
TE	Echo Time
TR	Repetition Time
VGT	Verb Generation Task
VK	Verbal Knowledge Test

3 INTRODUCTION

Stroke is a cerebrovascular disease causing rapidly developing disturbance of cerebral function, and is one of the leading causes of death in adults (Donkor, 2018). Stroke can occur during the entire lifetime including the fetal period and has a focused lifetime risk during the neonatal period (Kirton and deVeber, 2013). Stroke in children encompasses distinct disease states with diverse causality, mechanism, time of onset, mode of presentation and outcome.

The incidence rates of perinatal stroke vary; according to recent studies, the overall prevalence of perinatal stroke in term born children is as high as 1:1100 (Dunbar et al., 2020; Grunt et al., 2015; Laugesaar et al., 2007), but it is probably still an underdiagnosed condition (Dunbar et al., 2020). The increased awareness of perinatal stroke among neurologists and radiologists, and the increased availability of magnetic resonance imaging (MRI) has led to better recognition of the disease (Chalmers, 2005) and to its growing incidence in recent years (Dunbar et al., 2020). Similar growth in the incidence rate in the past decade has also been noted among young adults (Ekker et al., 2018; Kõrv et al., 2021).

Perinatal stroke causes lifelong morbidity, involving motor, cognitive and language impairment, and epilepsy (Dunbar and Kirton, 2018; Ferriero Donna M. et al., 2019; Hawe et al., 2020; Kirton and deVeber, 2013; Kuczynski et al., 2016; Lõo et al., 2018; Mercuri, 2003; Virani et al., 2022), and leads to academic difficulty (Jenks et al., 2009), behavioral problems and hyperactivity (Whittingham et al., 2014). Also, perinatal stroke causes a lifelong physical, mental, emotional and social burden for children and families and an economic burden for the society (Bemister et al., 2015). The lifelong individual, family, and societal burden of stroke is likely to be greater compared to adult stroke, as the infants and children who survive stroke will have many more years ahead living with disability. The key difference between children and adults is that childhood stroke results primarily in a changed ability to achieve, rather than lose, functional independence. However, there is a lack of studies for the first months of life providing information about prognosis and need for rehabilitation in children with perinatal stroke, and especially in those with presumed perinatal stroke without imaging for the acute stage of stroke.

In children, the brain is still actively developing the most basic functional networks compared to the highly optimised and matured adult brains (Kolb and Teskey, 2012). In adults, stroke leads to more permanent disabilities compared to childhood stroke (Szaflarski et al., 2014). At first glance, children with perinatal stroke mostly walk (Golomb et al., 2003), talk and interact like healthy children, but at closer evaluation they have various degrees of cognitive and motor deficits which are often combined (Chapman et al., 2003; Lõo et al., 2018). Moreover, cognitive deficits may emerge as late as school age with bain maturation (Westmacott et al., 2009). However, children's brain plasticity is limited (Bartha-Doering et al., 2019; Chapman et al., 2003; Ilves et al., 2014;

Knecht and Lidzba, 2016). Therefore, it is important to investigate how perinatal stroke changes typical maturation and organization processes of the developing brain.

Previous studies of perinatal stroke have evaluated mostly motor impairment and neurocognitive morbidity, while language has been less studied (Boardman et al., 2005; Craig et al., 2019b, 2019a; Dinomais et al., 2015; Golomb et al., 2003; Hawe et al., 2020; Husson et al., 2010; Kirton et al., 2008; Kitchen et al., 2012; Lõo et al., 2018; Wiedemann et al., 2020). Few studies have correlated functional MRI data to motor, cognitive and language abilities of children to find out prognostic MRI markers (Bartha-Doering et al., 2019; Carlson et al., 2019; Dinomais et al., 2012; Everts et al., 2010; François et al., 2021; Ilves et al., 2014; Jacola et al., 2006; Kornfeld et al., 2017; Lidzba et al., 2017a, 2017b; Ní Bhroin et al., 2021; Raja Beharelle et al., 2010; Saunders, 2014; Staudt, 2007; Woodward et al., 2019).

Perinatal stroke includes different subtypes with a different etiology, pathogenesis and location of damage (Dunbar and Kirton, 2018). The two major vascular types of perinatal stroke are arterial ischemic stroke (AIS) and periventricular venous infarction (PVI) (Kirton et al., 2008), which develop at different times of fetal life at different stages of brain's vascular development (Fluss et al., 2019). Many studies of perinatal stroke have focused on only the AIS subgroup of perinatal stroke (Boardman et al., 2005; Dinomais et al., 2015; Husson et al., 2010; Mercuri, 2003; Westmacott et al., 2009; Wiedemann et al., 2020), which is easy to recognize if seizures develop after birth, or have combined all the different vascular types of perinatal stroke under a single study group (Knecht and Lidzba, 2016; Sundelin et al., 2021; Niek E van der Aa et al., 2013). Outcome studies which combine all children with different vascular types of perinatal stroke to form a single study group (Ballantyne et al., 2008) offer unrealistic projections about the future of these patients for parents and physicians. The outcome of PVI is less explicitly studied (Hawe et al., 2020; Lõo et al., 2018) and the clinical outcome of children with PVI has been found to be similar with that of healthy controls (Carlson et al., 2019; Craig et al., 2019b; Lidzba et al., 2017a; Saunders et al., 2019). However, children with PVI have more motor problems compared to children with AIS (Lõo et al., 2018).

Radiological imaging allows to determine the vascular type and size of perinatal stroke, as well as to understand changes in the brain networks, compensatory mechanisms and brain plasticity after stroke. Advanced imaging like resting state and task based functional MRI (fMRI) and volumetric analysis, combined with evaluation of clinical outcome, may provide a novel understanding of brain plasticity in children with perinatal stroke. The knowledge of brain plasticity in different vascular types of stroke enables to identify patients with perinatal stroke who are at high risk to develop severe disability, and to evaluate their need for early rehabilitation and the possibilities of it.

4 LITERATURE REVIEW

4.1 Definition and classification of perinatal stroke

According to definition, perinatal stroke is “a vascular event causing focal interruption of blood supply, occurring between 20 weeks of fetal life through the 28th postnatal day, and confirmed by neuroimaging or neuropathology studies” (Fluss et al., 2019; Lynch, 2009; Lynch et al., 2002; Raju et al., 2007). Unlike definition of stroke in adults, the diagnosis of perinatal stroke is based on the neuroradiological confirmation of a vascular event rather than on the typical clinical presentation of a stroke, which is often absent in the case of perinatal stroke.

Stroke diagnosed between birth and four weeks of life is classified as neonatal stroke (Fluss et al., 2019). Neonatal stroke typically presents with seizures that develop a few days after birth (Dinomais et al., 2015; Nelson and Lynch, 2004). In some children hemiparesis or delayed developmental milestones appear after uneventful 4 weeks of the neonatal period and neuroimaging shows chronic stroke damage, while ischemic stroke is presumed to have occurred in the fetal period (Fluss et al., 2019; Ilves et al., 2016). Diagnosis in both neonatal and presumed antenatal perinatal stroke is based on MRI, which is done after symptoms have appeared.

Perinatal stroke can be classified according to the vascular type based on the criteria described by Kirton and coworkers (Kirton et al., 2008) on the basis of acute or chronic changes on MRI. The most common types of stroke are AIS and PVI (Ilves et al., 2016; Kirton et al., 2008; Kitai et al., 2016). Hemorrhagic and sinovenous thrombotic strokes are less common types of perinatal stroke in neonates (Clive et al., 2020; Kirton et al., 2008).

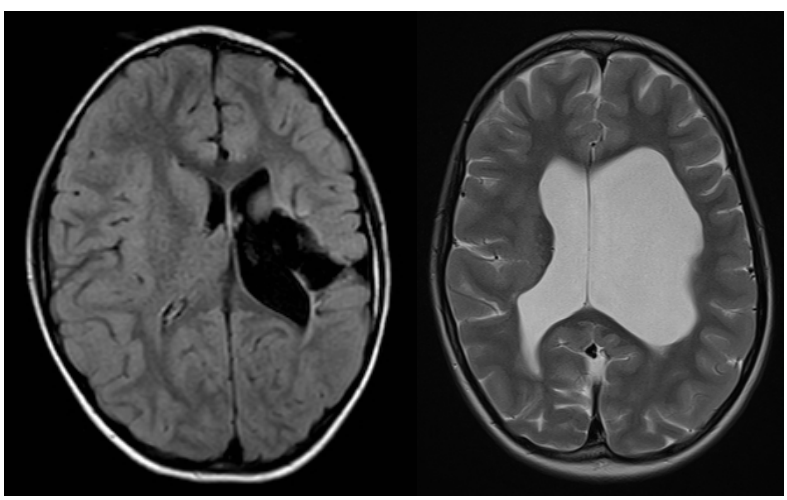

Figure 1. Lesions of the most common types of perinatal stroke: arterial ischemic stroke (left) on an anatomical axial FLAIR image and periventricular venous infarction (right) on T2 weighted axial brain MRI image

The representative lesion images of the two major vascular types of perinatal stroke, AIS and PVI, are shown in Figure 1.

4.1.1 Arterial ischemic stroke

AIS is caused by large artery occlusions and occurs around term birth (Fluss et al., 2019). Middle cerebral artery (MCA) is affected most frequently, accounting for 95% of single artery infarcts in newborns with AIS (Dunbar et al., 2020). Children with AIS usually have acute symptoms after birth (Lee et al., 2005a). MRI is performed to detect the underlying cause of neurological symptoms. Children with **neonatal AIS** are diagnosed early as seizures develop during the first days of life (Dinomais et al., 2015; Nelson and Lynch, 2004). Some patients with mild symptoms are probably missed and diagnosed after the neonatal period (Ilves et al., 2016) as **presumed perinatal AIS**. However, owing to better recognition of perinatal stroke by neonatologists and pediatric neurologists, age at diagnosis of presumed perinatal stroke has decreased over time (Dunbar et al., 2020).

Primary perinatal stroke lesions are usually unilateral (Dunbar et al., 2020; Grunt et al., 2015). AIS lesions are more common in the left hemisphere (Dunbar et al., 2020; Grunt et al., 2015), possibly due to abnormal hemodynamics and asymmetry of thoracic and aortic anatomy, which favor the migration of thrombus to the left hemisphere (Nelson and Blair, 2015; Parsa and Robert, 2013).

Neonatal AIS is more common in males compared to females (Dunbar et al., 2020; Golomb et al., 2004; Grunt et al., 2015; Laugesaar et al., 2007), whereas presumed perinatal AIS is more common in females (Dunbar et al., 2020; Laugesaar et al., 2007). The exact mechanism of this finding is not clear. One explanation for this is that females are more predisposed to thrombus formation and embolic risk (Parsa and Robert, 2013). Another explanation of female predominance in presumed perinatal stroke, both AIS and PVI, could also be increased mortality among male fetuses (Ilves et al., 2016).

4.1.2 Periventricular venous infarction

Neonatal PVI is caused by germinal matrix hemorrhage, which leads to perivenous thrombosis and periventricular venous infarction in preterm children born around gestational weeks 24-34 (de Vries et al., 2001; Fluss et al., 2019). However, some cases may already develop prenatally and are detected in preterm born children on the first day of life and diagnosed as neonatal PVI (de Vries et al., 1998).

Some fetuses with antenatal PVI stabilize and survive, showing no symptoms after uneventful term birth (Ilves et al., 2016). Term born children with **presumed PVI** are diagnosed after the perinatal period when hemiparesis or delayed developmental milestones appear. The problem of underdiagnosing all patients with PVI is universal, as mild cases of hemiparesis may be missed (Özduman et al., 2004). Presumed PVI is more common in females (Ilves et al., 2016).

4.2 Outcome in perinatal stroke

Motor and cognitive impairments are common in children with perinatal stroke. Combined deficits of motor, language and cognitive functions are found in 90% of children with AIS and in 53% of children with PVI (Lõo et al., 2018; Kirton and deVeber, 2013).

4.2.1 Motor networks and outcome

Perinatal stroke leads to damage of the developing sensorimotor system, including the motor and somatosensory tracts, which leads to contralesional spastic hemiplegia (Craig et al., 2019a; Kirton and deVeber, 2013, 2009; Lõo et al., 2018). Motor impairment depends on the relative volume of lesion on acute diffusion weighted imaging (Wiedemann et al., 2020) and on damage to the sensorimotor tracts: the descending corticospinal tract, the ascending thalamocortical tract and the posterior limb of the internal capsule (Mailleux et al., 2020). However, in cases of presumed perinatal stroke the acute phase MRI, including diffusion weighted imaging, is not available and the first MRI is done months after symptom onset. There is a lack of prognostic indicators for outcome in case MRI is performed in the chronic phase.

Lesions in the basal ganglia have a major impact on hand function (Feys et al., 2010; Holmefur et al., 2013; Holmström et al., 2010). Concomitant injury to the cerebral cortex, basal ganglia and posterior limb of the internal capsule in children with perinatal AIS has been shown to lead to unilateral motor impairment. However, if one or more of the mentioned structures in the corticospinal tract was spared, the outcome was normal (Mercuri et al., 1999; Boardman et al., 2005). Children with neonatal AIS and without impairment of the primary motor system (i.e. precentral gyrus, basal ganglia/thalamus, internal capsule) do not develop hemiparesis (Dinomais et al., 2015; Husson et al., 2010).

After perinatal unilateral injury the motor system is reorganized (Eyre, 2007; Staudt, 2007; Saunders, 2014). Recent studies have shown that not only the corticospinal tracts, but also structures remote from initial stroke damage, like the thalamus and corpus callosum, are involved in the case of neonatal AIS (Srivastava et al., 2019; Craig et al., 2019a). The knowledge of the reorganization and diaschisis of remote structures, like the sensory systems, basal ganglia, thalamus, cerebellum, and premotor and other motor network components, in different vascular types of perinatal stroke, as well as the knowledge of MRI changes affecting motor outcome are limited (Juenger et al., 2008).

4.2.2 Cognitive outcome

Outcome studies that cluster all children with perinatal stroke under a single study group (Ballantyne et al., 2008) may over- or underestimate their future cognitive outcome. It is important to consider the specific vascular type of stroke and its subtype, as cognitive deficit, language delay and behavioral

problems may vary (Wagenaar et al., 2018). Children with PVI seem to have lower risk of cognitive and behavioral disorders compared to children with AIS (Kirton et al., 2008; Lõo et al., 2018).

Almost all children with perinatal stroke achieve adequate communication and subnormal to normal intellectual abilities. Of children with AIS, 69% showed normal mental development, 21% had mildly delayed and only 10% had significantly delayed performance (Grunt et al., 2015). At school age approximately 60% of children exhibited mild forms of neurodevelopmental disabilities, which can be easily overlooked by parents and by professionals (Fluss et al., 2019). In a study by Ricci et al., the global intelligence of 78% of children with perinatal AIS was in a normal range at early school age (Ricci et al., 2008). In a study of children with neonatal AIS at the age of 7 years global intellectual deficiency was low in about 8%, however, a large subset of children showed low academic performance, which was associated with worse language skills (Chabrier et al., 2016). In a recent study children with perinatal stroke, combining both AIS and PVI, were shown to have lower memory, verbal and visual learning performances compared to healthy controls, which was not dependent on the side of stroke (Virani et al., 2022).

In children with AIS, poor academic perfomances and cognitive impairments tend to cluster with epilepsy and hemiparesis (Ballantyne et al., 2008; Fluss et al., 2019; Grunt et al., 2015; Kolk et al., 2011; Wagenaar et al., 2018). Combined subcortical and cortical damage leads to worse cognitive outcome compared to cortical or subcortical damage alone (Westmacott et al., 2010). Perinatal stroke, especially with subcortical lesions, e.g. in the basal ganglia and/or thalamus, had a larger negative impact on cognitive outcome than childhood stroke when children with unilateral AIS acquired at different ages were compared (Westmacott et al., 2010). No significant difference in cognitive outcome was found between the genders or lesion lateralities (Westmacott et al., 2010).

As maturation of the brain continues throughout childhood, development of later-maturing areas, which support higher level cognitive abilities, depend on the proper development of early-maturing areas (Gogtay et al., 2004). Brain plasticity enables to compensate for early-developing cognitive abilities in children with perinatal stroke. However, “exhausted” plasticity or the missed time window might limit subsequent development, causing poorer cognitive abilities in children with perinatal stroke later in childhood (Westmacott et al., 2010, 2009).

Westmacott and coworkers found that preschoolers with neonatal AIS did not differ from the normative sample in the cognitive test used, however, their cognitive deficits, notably in the domain of nonverbal reasoning, may have emerged later (Westmacott et al., 2009). Deficits in nonverbal reasoning, working memory and processing speed emerge only after 6 years of age in children with neonatal AIS (Westmacott et al., 2009).

In addition to cognitive problems, children with perinatal stroke often have behavioral problems, including hyperactivity (Whittingham et al., 2014).

4.2.3 Language

In an earlier Estonian study on different vascular types of perinatal stroke, language function was shown to be impaired in 68% of children (Lõo et al., 2018) and different studies in children with perinatal AIS show language delay in around 20–50% (Golomb et al., 2001; Lee et al., 2005b; Wagenaar et al., 2018). In children with AIS, language delay occurs usually together with motor and cognitive deficit (Wagenaar et al., 2018). Language impairment in combination with sensorimotor and cognitive problems is more common in children with AIS (81%) compared to children with PVI (53%) (Lõo et al., 2018).

4.2.3.1 Language networks

The classical language model consists of specific regions for language: Broca's area in the frontal lobe provides language production and Wernicke's area, located in the temporal lobe, provides language comprehension function. The two areas are connected with dorsal and ventral streams (Hickok and Poeppel, 2004). However, activations of the language production and comprehension network change during maturation (Weiss-Croft and Baldeweg, 2015) and the exact regions of Broca's and Wernicke's areas are difficult to define (Tremblay and Dick, 2016).

In the majority (92.5%) of right-handed adults language activation is lateralized to the left side (Knecht et al., 2000). Initially, the language networks are bilateral in young children and lateralize more to the left hemisphere with maturation, which progresses even in adolescence (Everts et al., 2008; Holland et al., 2001).

4.2.3.2 Language lateralization in stroke

In adults with matured brains and reduced plasticity (Boyke et al., 2008; Lövdén et al., 2013), the language network lacks the ability to relocate the language function after stroke, which leads to worse language outcome in adults compared to children (Szaflarski et al., 2014). In children the plasticity of the developing brain enables reorganization of language to the nonlesioned hemisphere (Bartha-Doering et al., 2019; François et al., 2019; Ilves et al., 2014; Lidzba et al., 2017a; Raja Beharelle et al., 2010). Moreover, the plasticity of the developing brain allows to use unconventional (intrahemispheric) brain regions for language, for example, congenitally blind individuals may employ the occipital cortices for language tasks (Bedny et al., 2011).

A recent study of healthy fetuses and infants found a rapid functional connectivity increase in resting state fMRI interhemispherically, but not intrahemispherically, in Broca's and Wernicke's areas during the third trimester, but no rapid increase in functional connectivity in the language areas in children up to 30 months of postnatal age (Scheinost et al., 2021). Thus, children's brain matures at high rate perinatally and language impairment along with latera-

lization is dependent on the vascular type of perinatal stroke and the corresponding time of damage (Brizzolara et al., 2002).

It is not clear how the language function changes and localizes anatomically after a specific vascular type of stroke in children. The outcome may depend also on the timing of stroke and on brain maturation. The language of children with perinatal stroke may be impaired more (Chapman et al., 2003) or less (Ilves et al., 2014) compared to children with childhood stroke at later age. Moreover, since in children with perinatal stroke cognitive impairment may become apparent only during school age (Westmacott et al., 2009), the time of cognitive outcome and language evaluation is extremely important.

Atypical lateralization of language comprehension appears to be more likely after AIS with cortical damage compared to PVI with periventricular lesions in the white matter (Brizzolara et al., 2002; Carlson et al., 2019; Lidzba et al., 2017a; Raja Beharelle et al., 2010). However, besides interhemispheric reorganization, also intrahemispheric reorganization, which is not measured by lateralization, is possible after perinatal stroke (Anderson et al., 2011; François et al., 2021).

4.2.3.3 Language lateralization and outcome

Information about associations between language outcome and lateralization in children with perinatal stroke, especially PVI, is limited, based on small groups, and has often been controversial.

Previous studies have suggested that in children with left-side perinatal stroke, the right hemisphere is "taking over" the language function (Jacola et al., 2006; Tillema et al., 2008), yet language impairment occurs in both right- and left-side childhood stroke (Avila et al., 2010; Chapman et al., 2003). Other authors propose that children with right-side lateralization do not achieve normal level of language after stroke (Bartha-Doering et al., 2019; Knecht and Lidzba, 2016; Raja Beharelle et al., 2010). Atypical lateralization of the language networks to the right hemisphere, caused by perinatal lesions, may result in delayed language development (Chilosi et al., 2005).

The majority of language lateralization and outcome studies of perinatal stroke focus on AIS (François et al., 2019) or lump together all vascular types of perinatal stroke (Northam et al., 2018; Raja Beharelle et al., 2010). In a study by Raja Beharelle and coworkers, language outcome did not differ significantly between the AIS and PVI subgroups of perinatal stroke. However, left lateralization in Broca's area, which was increased in the PVI group, was associated with better language outcome (Raja Beharelle et al., 2010).

4.2.4 Epilepsy

Epilepsy is common in children with perinatal stroke. It has been diagnosed in around 30% of children with perinatal stroke by teenage (Chabrier et al., 2016; Laugesaar et al., 2018; Wagenaar et al., 2018), however,epilepsy risk depends

largely on the vascular type and location of stroke (Laugesaar et al., 2018; Wusthoff et al., 2011). Epilepsy risk is higher among children with neonatal and presumed perinatal AIS (61% after 8.6 years of age) compared to presumed PVI (2%) (Laugesaar et al., 2018). Children with neonatal AIS have the highest risk of epilepsy, while presumed perinatal AIS involves more often severe epilepsy syndromes (Laugesaar et al., 2018). Meta-analysis has shown that in the case of short follow-up times, only 27% of children with perinatal AIS have had epileptic seizures (Rattani et al., 2019). However, if follow-up is long enough, up to teenage, children with neonatal AIS will have developed epilepsy (Laugesaar et al., 2018). The risk of epilepsy remains elevated even 20 years after stroke (Sundelin et al., 2021).

4.3 Neuroimaging in perinatal stroke

Neuroimaging, mainly ultrasound or MRI, is employed for the diagnosis of both fetal and neonatal stroke. MRI is a method in which a strong magnetic field and electromagnetic radio waves are used to excite atomic nuclei and an anatomical image is created using the corresponding relaxation signals, which are tissue dependent. The fMRI makes use of special MRI sequences for creating an image of functional activation of the brain.

MRI is used to confirm the diagnosis of perinatal stroke, as it defines the type and timing of lesion, and determines the etiology and prognosis in an optimal way (Raju et al., 2007). Neuroimaging enables to differentiate stroke from other neurological diseases with mimic symptoms, like asphyxia, as well as from congenital abnormalities and infectious diseases, including herpes and cytomegalovirus meningoencephalitis etc. As the availability of MRI increases, some children with presumed perinatal stroke and with chronic changes are detected during MRI imaging in children with mild neurological dysfunction.

Besides structural imaging used in clinical practice, also diffusion tensor imaging, volumetric analysis and fMRI can help to evaluate network damage and to investigate principles of possible brain plasticity in perinatal stroke.

4.3.1 Dynamic changes in magnetic resonance imaging

4.3.1.1 Dynamic changes in findings of magnetic resonance imaging in arterial ischemic stroke

At the acute stage of stroke diffusion weighted imaging on MRI shows diffusion restriction in the damaged tissue within a few hours. Low restriction coefficients persist for 6–10 days (Niek E. van der Aa et al., 2013), with larger diffusion changes indicating worse outcome (Srivastava et al., 2019). After a few days, a high signal on T2-weighted images with a loss of gray-white matter differentiation and a low signal on T1-weighted images are found in infarcted areas.

Loss of tissue and cystic change become visible after 2 weeks (Fluss et al., 2019). After stroke, within a few weeks, cerebral peduncle atrophy can be identified, indicating degeneration of the motor tract (Husson et al., 2010); diaschisis remote from the infarct in the thalamus, corpus callosum and cerebellum (Craig et al., 2019b, 2019a; Okabe et al., 2014) is associated with worse motor outcome.

At the chronic stage or in presumed perinatal AIS the porencephalic area is visible, involving cortical and subcortical gray matter in the MCA territory, with a size depending on the location of occlusion. At the chronic stage of AIS, the motor, language and other functions are reorganized and the change can be measured using fMRI and transcranial magnetic stimulation (Fiori and Guzzetta, 2015).

4.3.1.2 Dynamic changes in findings of magnetic resonance imaging in periventricular venous infarction

In the acute phase of PVI cerebral ultrasound shows germinal matrix and/or intraventricular hemorrhage and a fan- or globule-shaped increase in parenchymal echogenicity can be connected to the ventricle (Bassan et al., 2006; Elchalal et al., 2005; de Vries et al., 2001; eurUS.brain group et al., 2020). PVI usually evolves into unilateral cavitation within the periventricular white matter adjacent to the ventricular wall, and porencephaly due to cavitation is common after 1 or 2 months (eurUS.brain group et al., 2020).

On MRI images PVI is visualized as unilateral infarction in the periventricular area with porencephalic enlargement of the lateral ventricle, periventricular white matter gliosis and/or cystic changes with an intact cortex and relatively spared basal ganglia (Kirton et al., 2008). The residues of hemorrhage/hemosiderin can be visible in the area of the germinal matrix, periventricular lesion and/or ventricles on specific sequences (Fluss et al., 2019; Ilves et al., 2016; Kirton et al., 2008; Takanashi et al., 2003).

4.3.2 Preparation of children for magnetic resonance imaging

The methods of MRI, especially fMRI, are very sensitive to movement artefacts (Caballero-Gaudes and Reynolds, 2017; Friston et al., 1996). In young subjects MRI is usually performed under anesthesia to reduce movement artefacts. As anesthesia influences brain's resting state activity (Palanca et al., 2017), fMRI, especially task based paradigms requiring active participation, is limited in young subjects under the age of 6 years (François et al., 2019). Still, fMRI is used in the chronic phase of perinatal stroke for evaluation of outcome and scientific setting. Data about fMRI in infants is limited (Zhang et al., 2019) and even less available for the acute phase of perinatal stroke (Merhar et al., 2020, 2016; Seghier et al., 2004).

Preparation of subjects is important to eliminate any artefacts that would prevent the analysis of MRI data. FMRI data with movement artefacts can be

filtered out with a study specific maximum relative movement threshold (Power et al., 2014, 2012). Alternatively, movement components can be eliminated using a general linear model (Raja Beharelle et al., 2010) or independent component analysis for identifying motion artefacts (Griffanti et al., 2017).

MRI investigation can be frightening for young subjects, who often are afraid and do not want to enter the machine, or will abort the scan prematurely. A thorough preparation of children prior to fMRI scanning is helpful (de Bie et al., 2010). The preparation involves a description of the machine and the procedure, which is adjusted to the subjects' age.

The MRI scanner is a claustrophobic space enclosing the subject with an additional (head) coil, which reduces movement and the visual field. Lying completely still for a long time is challenging for everybody, especially children. Fortunately, a panic button is available, providing some comfort and a feeling of control.

During active scanning, loud knocking and changing sounds are produced, which may associate with broken equipment in children who feel insecure inside the machine. Additionally, namely the clinical environment in hospital may be associated with unpleasant and uncomfortable experiences for children, especially those with perinatal stroke.

To obtain some useful data from scans aborted by the participant before completion, our protocol started with essential sequences and continued with more demanding ones. In the case of an aborted scan or motion artefacts, a rescan was scheduled and performed when possible.

4.3.3 Volumetric analysis in perinatal stroke

The brain of children with perinatal stroke is deformed by stroke lesion and by the brain's reorganization due to focal damage. Diaschisis (Carrera and Tononi, 2014) and other reorganization and compensatory changes of brain regions remote from the damage occur in anatomical MRI. These differences in brain structure can be compared between different individuals and groups, using segmentation of brain structures and volume measurements. Subcortical structures are essential in different brain functions like performance of motor and cognitive tasks (Middleton and Strick, 2000). Volumetric analysis reveals plastic reorganization of the brain following perinatal stroke damage and can be perfomed on the basis of data obtained during anesthesia. The results can be used for predicting outcome in children with presumed stroke in cases where initial images are lacking.

In children with pediatric stroke the thalamus was found to be the most common location of acute diaschisis (Kirton et al., 2016). Primary perinatal stroke lesions typically spare the cerebellum and thalamus, however, previous studies have shown measurable structural alterations due to their high connectivity to damaged brain regions (Craig et al., 2019b, 2019a). Ipsilesional thalamic volume was shown to be reduced in both children with AIS and PVI, without correlation with motor function; contralesional thalamic volume was

increased in children with AIS compared to PVI and in healthy controls (Craig et al., 2019a). In another study atrophy in the mediodorsal thalamus and cerebellum was correlated with contralesional hand performance in children with neonatal AIS (Dinomais et al., 2016).

4.4. Functional magnetic resonance imaging

4.4.1 Functional magnetic resonance imaging method

FMRI, which is the neuroimaging method used in the current study, is based on blood-oxygen-level-dependent imaging sequences. It was developed to replace investigation of metabolic activity by positron emission tomography imaging without the use of radioactive tracers. Increased metabolism in the active brain tissue requires more oxygen and increases the local blood flow providing oxygen. The blood-oxygen-level-dependent signal in fMRI measures the concentration of oxygenated hemoglobin and provides an indirect measure of brain activity. This magnetic property of hemoglobin was first described already in 1936 (Pauling and Coryell, 1936), but was applied in MRI as the blood-oxygen-level-dependent signal much later, in 1991 (Bandettini, 2012). The fMRI was validated using positron emission tomography imaging, which was the golden standard at that time. Today, hybrid positron emission tomography/MRI systems have been introduced, which enable to simultaneously combine fMRI and functional positron emission tomography scans (Rischka et al., 2018), but their use is still unethical in children because of radiation.

Indirect measurement of brain activity through the cerebral blood flow limits both the spatial and temporal resolution of fMRI. Compared to electroencephalography, fMRI provides better spatial resolution and allows imaging of the entire brain. The temporal resolution of fMRI is limited by a 2 to 6 sec delay from activity in the brain tissue to the response in the blood flow (Hirano et al., 2011). The blood flow is individually regulated on the scale of a few millimeters, limiting the spatial resolution of fMRI. Additionally, the blood-oxygen-level-dependent signal is susceptible to the magnetic field's inhomogeneity and movement artefacts. Movement artefacts originate in both physiological movement, like heartbeat or breathing, and the patient's ability to lie still in the MRI scanner.

4.4.2 Paradigm types in functional magnetic resonance imaging

The fMRI experiments can be divided into two major types (Maheshwari et al., 2021; Smitha et al., 2017): task based sequences, during which the subject has a specific task to perform, and resting state sequences without a specific task (Biswal, 2012). Compared to resting state sequences, fMRI task based sequences are easier to interpret, as the link between the function and the activation pattern is known. However, task based sequences require additional cooperation and active participation from the subject and are more difficult to perform compared to resting state sequences.

4.4.2.1 Task based functional magnetic resonance imaging

A task based sequence is usually structured into blocks of tasks that are repeated several times to attain average activation during multiple executions of a single task (Engström et al., 2004). There can be numerous types of tasks, which may be combined with control tasks to activate a certain area in the brain and to provide definite indication for the researchers that the subject is cooperating. The control task may be, for example, pushing a button after a stimulus or command.

The number of task based fMRI tasks is virtually unlimited. The tasks are often based on an auditory or visual stimulus requiring the subject's attention. They activate specific regions in the brain. The response to the stimulus can be covert, without movement, which eliminates possible movement artefacts (Engström et al., 2004). The response can also be overt such as another action (pushing a button).

Auditory stimulation tasks with covert response with the use of an integrated audio communication system are easy to perform compared to visual tasks that require specific screens. Auditory stimulation has been shown to produce more reliable language networks compared to visual presentation (Holland et al., 2001). To obtain a complete understanding of how a function activates the brain, use of multiple types of tasks is necessary (Engström et al., 2004; Wilke et al., 2010).

4.4.2.2 Resting state functional magnetic resonance imaging

The networks activated in a brain resting between fMRI tasks were initially considered to be noise and were removed by averaging them to increase the task based signal. However, it was noted later that the noise occurring during the resting state of the brain had specific patterns, which can be analysed (Biswal et al., 1995).

Different functional networks are spread over the different parts of the brain and are activated simultaneously and spontaneously in a "resting" brain without a specific task or stimulus (Fox and Raichle, 2007). In a continuous state the brain cannot be deactivated, as it has to immediately respond to external environmental changes and stimuli.

During resting state fMRI the subject is instructed to either have the eyes open or closed and not to think about anything particular. The brain is active and different thoughts and functions are activated spontaneously. For example, the subject may recall experiences or plan future tasks, process the sound of the MRI scanner, gaze at the inside of the scanner etc.

During short resting state fMRI, usually between 5 and 20 minutes in the case of children, the brain does not receive specific stimulation and not all networks may be spontaneously activated, or they are less activated and missed during analysis. Although longer scanning could minimize this bias, the likelihood of motion artefacts will be increased.

At the time of spontaneous thoughts, different areas of the brain are activated simultaneously, which serve as functional networks. Independent component analysis (ICA) enables to divide an fMRI dataset into a set of time courses and a set of spatial maps, which can jointly best describe underlying hidden components or networks (Beckmann et al., 2005). Probabilistic ICA assumes that there is a limited number of statistically independent non-Gaussian components mixed with Gaussian noise. Probabilistic ICA does not have a pre-specified model, such as the generalized linear model (GLM), and it estimates temporal patterns from input data. Therefore, resting state fMRI data can be analysed using probabilistic ICA, which does not require the unknown pattern of spontaneous activation.

The basic functional networks, like visual, auditory and motor networks, are structured similarly in all brains (Beckmann et al., 2005; Smitha et al., 2017), but they may have different robustness and varying occurrence (Muetzel et al., 2016). Functionally relevant resting state networks have been described previously in children (de Bie et al., 2012; Muetzel et al., 2016; Thornburgh et al., 2017; Wylie et al., 2014). The resting state networks involved in sensory related processing, like auditory and visual networks and motor networks, are similar in children and adults (de Bie et al., 2012). Resting state functional networks mature and evolve with age from birth to adulthood (Grayson and Fair, 2017). If and how similar functions are combined or divided in resting state networks depends also on the specific group and on the parameters of analysis (Thornburgh et al., 2017).

The auditory resting state network is a bilateral network in the temporal and cingulate gyrus involved in language processing (de Bie et al., 2012). A more leftward lateralized network in the inferior frontal gyrus, pars triangularis, orbitofrontal cortex, paracingulate area, posterior cingulate cortex, temporal lobe and precuneus is reported as the language resting state network (de Bie et al., 2012).

There are multiple visual processing resting state networks. The primary visual network is located in the striate and peristriate cortex, the secondary visual network is located in the lateral and superior occipital regions associated with the ventral visual stream. The dorsal visual stream network contains the supramarginal gyrus extending to the superior parietal cortex and inferior temporal gyrus (de Bie et al., 2012).

The somatosensory motor networks are located in the pre- and post-central gyrus including the somatomotor and somatosensory cortex and may involve also the thalamus and cerebellum (de Bie et al., 2012). The somatosensory and motor networks may be separate networks in children and combined networks in adults (Thornburgh et al., 2017).

The task positive resting state networks (Cole et al., 2010) involved in salience processing, goal directed behavior, attention and cognitive flexibility are connected to the cingulo-opercular network and activate the inferior frontal gyrus, insular cortex and cingulate gyrus (de Bie et al., 2012).

The default mode network, activated during the resting state (Raichle et al., 2001), is thought to activate during self-directed thought or introspection among others and is active also during sedation (Greicius et al., 2008); it is affected by various diseases (Mohan et al., 2016). The default mode network develops during childhood and adolescence and becomes more integrated with age (de Bie et al., 2012; Fan et al., 2021; Horowitz-Kraus et al., 2017). The default mode network in children is divided into a various number of separate resting state networks, which are overlapping in the precuneus (de Bie et al., 2012). The posterior part of the default mode network activates the posterior cingulate cortex, precuneus and angular gyrus bilaterally and is the most robust part of the default mode network in children (Muetzel et al., 2016; Thornburgh et al., 2017). Another frontal component is located in the bilateral superior frontal gyrus, cingulate gyrus and precuneus. An additional component of the default mode network activates the posterior cingulate cortex and precuneus, the parahippocampal cortices and inferior temporal gyrus (de Bie et al., 2012).

4.4.3 Resting state functional magnetic resonance imaging in perinatal stroke

With advances in the methods of analysis and in experience with the application of resting state fMRI, the number of resting state fMRI studies on childhood stroke and perinatal stroke has increased. Many of the resting state fMRI studies in children involve a healthy population (de Bie et al., 2012; Jiang et al., 2018), which provides an important baseline for interpretation of the data of perinatal stroke. The number of resting state fMRI studies in children with perinatal stroke is limited, but has increased after our first study in 2016 (Carlson et al., 2019; Dinomais et al., 2012; François et al., 2019, 2016; Kornfeld et al., 2017, 2015; Ní Bhroin et al., 2021; Saunders et al., 2019; Saunders, 2014; Woodward et al., 2019). The scarcity of resting state fMRI findings has probably been due to the complexity of their analysis and interpretation.

Some recent studies have found associations between changes in resting state functional connectivity and outcome in children with perinatal stroke. The ICA analysis of resting state fMRI data of subjects with perinatal stroke is limited (François et al., 2019, 2016; Kornfeld et al., 2017), focusing on the default mode network and the frontoparietal network.

The majority of resting state fMRI analyses in children with perinatal stroke are based on functional connectivity between specific regions of the brain and its lateralization. Language outcome and functional connectivity have been studied earlier (Carlson et al., 2019; François et al., 2016, 2019). François et al., reported a correlation between increased functional connectivity on the right hemisphere and language outcome, however, their study group was small. The connectivity of the motor functional network has also been analysed using resting state fMRI (Dinomais et al., 2012; Saunders et al., 2019); there was a difference in connectivity between children with AIS and PVI, and similarity in connectivity between children with PVI and healthy controls, but no correlation

was found with motor outcome in patients with perinatal stroke. However, one study focusing on children with PVI evaluated also the thalamus and found a difference in connectivity in the sensory motor functional network between children with PVI and healthy controls, and correlation between connectivity strength and motor outcome (Woodward et al., 2019). In a study of mostly childhood AIS with only three perinatal stroke subjects (Kornfeld et al., 2017) ICA, followed by frontoparietal network connectivity analysis, showed a difference between children with AIS and healthy controls; connectivity was also correlated with cognitive outcomes when the groups of perinatal stroke and control were combined.

4.5 Summary of the literature review

Perinatal stroke is a rare disease with varying vascular origin. It has heterogenous outcome and may lead to lifelong motor, cognitive and language impairments of various degrees depending on the vascular type and size of lesion. As most previous studies had small study groups due to the low incidence of perinatal stroke, the authors investigated a single stroke type or combined several vascular stroke types under a single group. Therefore, the data about differences in brain plasticity and long-term outcome between the different vascular types of perinatal stroke are limited.

Brain MRI is used to evaluate the vascular type and size of brain damage in children with perinatal stroke. Changes on diffusion weighted images during the first weeks after neonatal stroke can predict long-term outcome in children with perinatal stroke. In the case of presumed perinatal AIS and PVI, MRI is not performed during the acute phase and so radiological markers to predict long-term outcome are lacking.

Previous studies have shown volume changes in specific brain areas remote from the primary stroke lesion, e.g. in the thalamus and cerebellum of children with perinatal stroke. How volume changes in the basal ganglia and thalamus in different vascular types of perinatal stroke are associated with motor outcome needs further research.

Use of fMRI enables to gain a better understanding of brain's functional reorganization in children with perinatal stroke. Resting state fMRI allows to investigate general changes in brain network activation, which are not limited to a specific task, in different vascular types of perinatal stroke.

Language lateralization after perinatal stroke has been studied previously by using task based fMRI. However, previous research on language lateralization and language outcome in patients with perinatal stroke is limited, combines different vascular types of stroke or is only focused on children with AIS.

5 AIMS OF THE STUDY

The general aim of the thesis was to investigate the reorganization and plasticity of the brain in children with perinatal stroke. We hypothesized that different vascular types of perinatal stroke, i.e. AIS and PVI, have different patterns of brain's structural organization and network reorganization, which leads to different outcomes in patients with perinatal stroke.

The specific aims were:

1. To evaluate the volumes of the ipsi- and contralesional basal ganglia, amygdala, thalamus, and hippocampus following perinatal ischemic stroke in relation to hand motor function in children with a history of perinatal AIS and PVI and to compare the volumes of the subcortical structures in children with perinatal stroke and in healthy controls. (Substudy I)
2. To identify differences in resting state networks and cognitive development in children with perinatal AIS and PVI and to compare the obtained data with the corresponding data for healthy controls. (Substudy II)
3. To evaluate differences between language lateralization in task based fMRI and language outcome in term born patients with different vascular types of perinatal stroke, i.e. perinatal AIS and presumed PVI, and to compare these findings with those obtained for healthy controls. (Substudy III)

6 SUBJECTS AND METHODS

6.1 Subjects

The study populations were recruited from the Estonian Pediatric Stroke Database according to the substudy-specific inclusion and exclusion criteria.

6.1.1 Estonian Pediatric Stroke Database

The Estonian Pediatric Stroke Database was launched as an epidemiological study and subjects were entered from 1994 to 2003 retrospectively and since 2004 prospectively (Laugesaar et al., 2007; Ilves et al., 2016; Lõo et al., 2018; Laugesaar et al., 2018).

Before inclusion of a patient in the Estonian Pediatric Stroke Database, three pediatric neuroradiologists, who are blinded to findings of clinical outcome, independently review radiological images, confirm perinatal stroke and classify perinatal stroke according to the vascular type using the criteria developed by Kirton et al. (Kirton et al., 2008) and modified later by Ilves and co-workers (Ilves et al., 2016, 2014).

The exclusion criteria for the Estonian Pediatric Stroke Database are absence of confirmative cranial imaging (MRI); other diseases involving the central nervous system as suggested on the basis of neuroimaging and clinical findings: 1) severe birth asphyxia including watershed infarction; 2) infectious diseases including meningoencephalitis; 3) metabolic and mitochondrial disturbances and diseases including hypoglycemia and kernicterus; 4) tumors; 5) other cortical malformations or congenital anomalies.

6.1.2 Inclusion criteria (Substudy I)

Patients from the Estonian Pediatric Stroke Database were included in the study group, if they fulfilled all of the following inclusion criteria: 1) confirmed diagnosis of neonatal AIS, presumed perinatal AIS or presumed PVI; 2) birth ≥ 36 gestational weeks; 3) follow-up 3D T1 weighted image from 3T MRI performed at age 6-18 years without anesthesia, or from clinical 3T MRI with 3D T1 weighted image performed under anesthesia at age < 6 years; 4) affected hand function measured by Assisting Hand Assessment (AHA) test between 3.5 and 13 years of age.

The initial study group for substudy I consisted of 42 patients with perinatal stroke; eight of them were excluded due to artefacts in MRI or lack of AHA testing. The final study group for Substudy I consisted of a total of 34 subjects with perinatal stroke (7 children had neonatal AIS, 9 children had presumed perinatal AIS and 18 children had presumed PVI). All of the perinatal stroke subjects were aged 6-18 years except for one subject in whom clinical MRI was performed at 4.9 years of age with anesthesia. The majority of the subjects with perinatal stroke, 12/16 (75%) children with AIS and 12/18 (67%) children with

PVI, had unilateral left-hemisphere stroke lesion. Healthy controls (n=42) were 8-18 years old.

6.1.3 Inclusion criteria (Substudy II)

Term born children from the Estonian Pediatric Stroke Database were included in the study group, if they fulfilled all of the following inclusion criteria: 1) radiologically documented presumed or neonatal unilateral left-hemisphere AIS or presumed PVI ; 2) birth ≥ 36 gestational weeks; 3) age between 7 and 17 years; 4) capability to perform the MRI protocol without sedation.

Out of the 80 subjects in the Estonian Pediatric Stroke Database at the time, 46 children met the inclusion criteria for resting state fMRI study, i.e. substudy II. When contacted by phone, 10/24 (42%) children with unilateral left-side AIS, 11/22 (50%) children with unilateral left-side PVI and 25 healthy controls agreed to participate in the study and were able to do it. Ten children, among them children with perinatal stroke (AIS n = 3, PVI n = 1) and healthy controls (n = 6), were excluded due to aborted MRI scans or artefacts found during analysis. The final study group for substudy II consisted of 36 children (aged 7.6-17.9 years), among them 7 with AIS, 10 with PVI and 19 age and gender matched controls.

6.1.4 Inclusion criteria (Substudy III)

Patients from the Estonian Pediatric Stroke Database were included in the study group of substudy III, if they fulfilled all of the following inclusion criteria: 1) confirmed diagnosis of unilateral left-hemisphere neonatal AIS, presumed perinatal AIS or presumed PVI; 2) birth ≥ 36 gestational weeks; 3) ability to cooperate and perform fMRI tasks; 4) age 7–18 years at the time of fMRI investigations; 5) one main language in the family.

The study group consisted of 9 children with AIS, 12 children with PVI and 34 healthy controls. Four of the controls in the initial study group lacked at least one successful fMRI task without artefacts and were excluded from the final study group. The final study group consisted of children who had at least one successfully completed fMRI language task: 9 children with AIS (age 9.9–17.3 years), 12 children with PVI (age 7.3–15.9 years) and 30 healthy controls (age 8.1–18.7 years).

6.1.5 Control group (Substudies I–III)

The healthy control group for all the substudies (I–III) was recruited from among children of the hospital's staff members and their acquaintances and from among their classmates and friends attending regular school without learning difficulties and without known psychiatric or neurological diseases. The healthy control group was formed of term born children who were age and gender matched with children with perinatal stroke.

6.2 Neurodevelopmental assessment

6.2.1 Global outcome (Substudies I-III)

Neurodevelopmental outcome in children with perinatal stroke was evaluated by pediatric neurologists according to the criteria of Pediatric Stroke Outcome Measurement (PSOM) (Kitchen et al., 2012), a stroke-specific outcome measure, which scores right and left sensorimotor, language, cognitive and behavioral performance. PSOM provides a deficit score ranging from 0 to 2 (0: no deficit, 0.5: mild deficit, normal function, 1: moderate deficit, impaired function, and 2: severe deficit, missing function). The maximum total PSOM score, i.e. the sum of the five subscales, is 10. Moderate to severe unilateral sensorimotor deficit corresponded to definition of spastic hemiparesis: unilateral increased muscle tone and pathological reflexes, resulting in an abnormal pattern of movement and posture (Cans, 2000).

6.2.2 Cognitive and language evaluation (Substudies II and III)

Cognitive performance was evaluated by a clinical psychologist using the Kaufman Assessment Battery for Children II (Kaufman and Kaufman, 2004).

The battery contains 3 main cognitive indices: Fluid-Crystallized Index (FCI) – a general measure of cognitive ability including acquired knowledge; Mental-Processing Index (MPI) – a measure of mental processing ability that excludes measures of acquired knowledge; Nonverbal Index (NVI) – a general measure of nonverbal abilities. In addition, five subscales, i.e. sequential and simultaneous processing, learning, planning and knowledge, were used to assess broad abilities in specific domains.

Children's language related abilities were assessed in detail using three language subtests from the Kaufman Assessment Battery for Children II, resembling the fMRI language tasks used in substudy III. Expressive vocabulary test (EV) measures the verbal knowledge and expressive language skills of the child. Verbal knowledge test (VK) is based on the child's receptive language skills and measures acquired knowledge. Riddles test (RID) measures the verbal comprehension and verbal reasoning ability of the child.

The range of possible index and subscale scores is 40 to 160 (mean 100, standard deviation (SD) 15). The scaled scores of the subtests range from 1 to 19, and have a mean of 10 and an SD of 3.

The clinical psychologist performing the evaluation was blinded to the subjects' vascular type of perinatal stroke, or to the fact if the child was in the perinatal stroke group or in the healthy control group.

6.2.3 Motor evaluation (Substudy I)

Motor function evaluation was performed only in stroke patients. The affected hand function was evaluated by a pediatric neurologist using the AHA test battery (Krumlinde-Sundholm et al., 2007; Krumlinde-Sundholm and Eliasson,

2009). AHA measures how effectively children with unilateral impairment spontaneously use their affected hand during bimanual activities. AHA is based on Rasch analysis and provides a score from 0 to 100, with higher score indicating better ability.

The bimanual function was classified using the Manual Ability Classification System (MACS), which measures children's self-initiated motor ability during daily activities (Eliasson et al., 2006). It contains five levels; from successful handling of objects at level I to complete inability to handle objects at level V.

6.3 Radiological imaging: magnetic resonance imaging

6.3.1 Preparation of subjects

All subjects were prepared for MRI by appropriately introducing the procedure according to age. For younger subjects, a miniature toy MRI scanner was used to introduce the scanner through play. The MRI protocol and tasks were explained in detail and practiced to evaluate the children's ability to perform the tasks before the study.

6.3.2 Magnetic resonance imaging protocol (Substudies I–III)

MRI data was acquired using a 3 Tesla Philips Achieva MR scanner with a 8-channel SENSE head coil (Philips Medical Systems, Best, The Netherlands) without anesthesia, employing a fixed imaging protocol. Anesthesia was used in one child with the clinical need for MRI at the age of 4.9 years (substudy I).

6.3.2.1 Anatomical T1 sequence (Substudies I–III)

The protocol started with 3D anatomical T1 weighted images, followed by fMRI sequences. The T1 weighted sequence had 1mm isotropic voxels, 256x256 mm field of view. It was obtained using fast field echo with repetition time (TR) = 8.2 ms and echo time (TE) = 3.8 ms. The T1 weighted image was used in substudies II and III for matching all subjects to a common template (MNI-152 2mm standard space, Montreal Neurological Institute, Montreal, QC, Canada) and in substudy I for volume analysis.

To determine the extent of damage in children with perinatal stroke, a 3D fluid-attenuated inversion recovery (FLAIR) image sequence was used, with 1mm isotropic voxels with 256x256 mm field of view, TE = 289 ms, TR 4.8 s and inversion time (TI) = 1.7 s.

6.3.2.2 Lesion size classification (Substudy III)

AIS is classified according to the location of occlusion (Ilves et al., 2016; Kirton et al., 2010, 2008). Proximal M1 infarction (PMI) involves proximal MCA including lateral lenticulostriate arteries, leading to infarction of the basal ganglia and the distal cortical MCA territory. Distal M1 infarction (DMI) compromises the artery distal to lateral lenticulostriate, which spares the basal ganglia while infarcting the distal MCA territory. Anterior trunk (AT) infarction is infarction of the superior MCA division, including the cortex of the frontal lobe and the anterior temporal lobe. Posterior trunk (PT) infarction is infarction of the inferior MCA division, which includes the cortex of the parietal and posterior temporal lobes. AT and PT type AIS lesions are usually smaller in size compared to PMI and DMI type AIS lesions.

Perinatal stroke lesions were dichotomized into small and large lesions. AT and PT type AIS lesions were considered small AIS stroke lesions and PMI and DMI type AIS lesions were considered large AIS stroke lesions (Lõo et al., 2018; Westmacott et al., 2009). PVI lesions with involvement of several lobes were considered to be large lesions (Laugesaar et al., 2018; Lõo et al., 2018).

6.3.2.3 Resting state functional magnetic resonance imaging (Substudy II)

A 6-minute resting state fMRI scan with 120 time points, each containing 50 axial T2*- weighted slices, with isotropic 3mm voxel size covering the whole head, was used. The sequence used a fast field echo single shot echo-planar imaging -blood-oxygen-level dependent sequence with TR = 3000 ms, TE = 35 ms and 230x230 mm field of view. The participants were asked to stay awake and keep their eyes open during the resting state fMRI scan.

6.3.2.4 Task based functional magnetic resonance imaging (Substudy III)

Two task based fMRI sequences with language and motor tasks were performed (substudy III). According to the periodic block design, 40-sec language tasks and 40-sec motor tasks were performed alternately five times each, with a total sequence length of 100 time points, during 6 min and 40 sec. The functional images were obtained using a fast field echo single shot echo-planar imaging blood-oxygen-level dependent sequence with TR = 4010 ms, TE = 35 ms, 42 axial slices, 128x128 matrix and 1.5x1.5x3 mm voxels covering the entire brain. The language tasks were presented in an auditory form by male voice, using headphones. The motor tasks with self-paced finger tapping to the thumb were used as control to confirm the subject's cooperation.

During the first fMRI test, a verb generation task (VGT) was combined with the right hand finger tapping control task. In the second fMRI test, the sentence comprehension task (SCT) was combined with left hand finger tapping as control.

6.3.2.5 Verb generation task

During VGT the subjects were presented with a series of nouns and instructed to think about an action associated with each noun. For example, if the noun "pencil" was presented, the subject might generate verbs like "draw", "write" or "sharpen". The VGT was designed to activate the expressive language area in the frontal lobe (Broca area), as described earlier (Holland et al., 2001; Engström et al., 2004; Wilke et al., 2006; Tillema et al., 2008b).

6.3.2.6 Sentence comprehension task

The SCT was designed to activate the receptive language area in the temporal lobe (Wernicke area); the subjects were presented with sentences and instructed to tacitly decide, if the sentence was correct or incorrect. For example, the correct sentence might be "shoes are worn on the feet", while the incorrect sentence would be "shoes are worn on the head". The ratio of the right to wrong sentences was 7:1.

6.4. Methods of image analysis

6.4.1 Quality control and anonymization

The images were visually inspected for motion and other imaging artefacts. When necessary, sequences with poor quality were excluded from further analysis or, when possible, the scan was rescheduled. Children who initially were afraid to undergo scanning or were not able to perform it, passed the rescheduled scanning successfully.

All MRI scans including healthy children were stored in DICOM format in the population based Estonian Medical Picture Archive (www.pacs.ee) (Aavik et al., 2007) and were evaluated and reported by a neuroradiologist to detect incidental findings even in healthy children. Additionally, all MRI scans were anonymized and stored on a hospital based custom database server created for scientific purposes. The anonymized MRI dataset was transferred securely to the University of Tartu High Performance Computing Centre where computationally demanding analysis was performed.

After each processing step in the workflow, described below, visual quality control was done and when necessary, individual manual corrections and parameter optimization were performed.

6.4.2 Anatomical magnetic resonance image analysis (Substudy I)

To analyse volume differences between the study groups, only the anatomical 3D T1 weighted sequences were used. All images of the right-side lesions were mirrored along the x-axis and all lesions were analysed as left-side lesions.

6.4.2.1 Voxel based morphometry

The FSL's voxel based morphometry workflow was employed to analyse differences in the amount of gray matter between the AIS, PVI and control groups. A left-right symmetric gray matter template was created from a randomly sampled equal-size subset for each study group. Anatomical images from all subjects were registered to the study specific template and a voxelwise general linear model was used to identify differences in gray matter between the AIS, PVI and control subjects.

6.4.2.2 Segmentation and volume measurement

Subcortical gray matter volumes were measured using semiautomatic segmentation. Initial segmentation was done automatically using the FSL's FIRST tool, followed by manual correction using the FSL's FSLeyes. Quality control and manual correction with a random order of subjects were performed two times with a 2-week interval. Lesioned areas were excluded from segmentation using lesion maps. The FSL's tool Fslstats was used to measure the volume of segmentations. The segmented volumes were normalized by individual volumetric *scaling factor* generated by using the FSL's SIENAX tool (Smith et al., 2002), which enables to convert the subject's brain volume to MNI152 standard space volume which is comparable for the subjects (Kijonka et al., 2020). The scaling factor is a measure of subject's head size.

6.4.3 Resting state functional magnetic resonance image analysis (Substudy II)

The ICA of the resting state fMRI images was performed using the FMRIB software library's (FSL) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) MELODIC tool. The workflow included motion correction, brain extraction, spatial smoothing and temporal filtering of blood-oxygen-level dependent images. To perform groupwise analysis in a standard space, the subject's fMRI images were registered to their T1 images, which were subsequently registered to the MNI-152 standard space (Jenkinson and Smith, 2001; Jenkinson et al., 2002).

The resting state data for all subjects were temporally concatenated for probabilistic ICA. The dataset was decomposed into 30 independent components and visually evaluated to identify known resting-state networks from among artefact components.

Differences between the AIS, PVI and control groups in the known resting state networks were calculated using FSL dual-regression and randomized tools. The obtained statistical maps were corrected for threshold-free cluster enhancement (TFCE) and false discovery rate (FDR).

6.4.4 Task based functional magnetic resonance image analysis (Substudy III)

The task based fMRI sequences were analysed to find out individual lateralization of language activation and differences in language activation between the study groups.

During pre-processing of the fMRI data, thermal noise was first removed, after which the motion and physiological components, calculated by the FSL's MELODIC tool, were manually classified (Griffanti et al., 2017) and regressed out using the FSL's *fsl_regfilt* script.

Individual language activations during both VGT and SCT were calculated using the FSL's FEAT tool (Woolrich et al., 2004, 2001). The fMRT images were registered through the 3DT1 weighted sequence to MNI-152 standard space (Jenkinson and Smith, 2001; Jenkinson et al., 2002). Manual correction and lesion masks were used during registration when necessary. A linear model corresponding to the periodic language stimulation of the task paradigm was employed to calculate language activation. Activation was TFCE corrected.

Higher level FEAT analysis was performed to find differences in language activation between the study groups and the effect of age, gender, language and cognitive outcome on language activation.

6.4.4.1 Lateralization index

Language task specific activation was compared between the task relevant regions of interest (ROI) in the two brain hemispheres. Comparison was based on lateralization index (LI).

LI is defined as $LI = \frac{L-R}{L+R}$ where L is the number of activated voxels in the left hemisphere and R is the number of activated voxels in the right hemisphere within a specific ROI. LI has values between -1 and 1. Positive LI values indicate left hemisphere lateralization and negative values, right lateralization of activation. A LI within 2 SD of the control group's mean LI was considered typical (T) and reorganized (R) otherwise (Lidzba et al., 2017a).

LI was calculated using the LI-toolbox in SPM12 (Penny et al., 2006) running on Matlab by applying the bootstrap/histogram analysis method (Wilke and Lidzba, 2007; Wilke and Schmithorst, 2006).

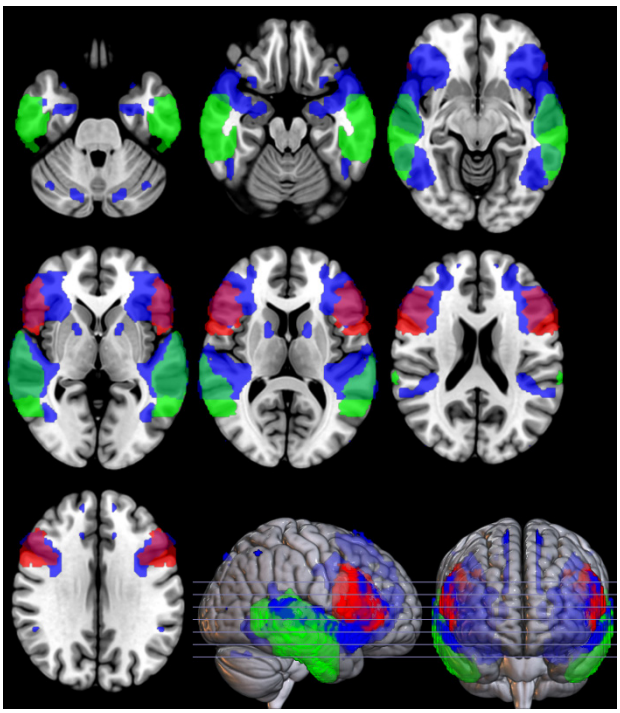

Figure 2. Regions of interest for calculation of lateralization index (LI) in Substudy III. Broca area in red, Wernicke area in green and mean fMRI activation in blue.

Activation in certain ROIs was compared for the two brain hemispheres. Language LI was calculated for three ROIs as shown in Figure 2.

The traditional language areas, i.e. Broca and Wernicke, were defined using atlases in FSL. For the Broca ROI, Brodmann areas 44 and 45 in the left and right hemispheres, based on the *Juelich probability atlas* (Amunts et al., 1999), were combined and thresholded at 20 using *fslmaths*. The Wernicke ROI was generated by combining the superior temporal gyrus (anterior and posterior divisions), the middle temporal gyrus (anterior and posterior divisions, temporo-occipital part) and the inferior temporal gyrus (anerior and posterior divisions), based on the *Harvard-Oxford cortical probability atlas* (Desikan et al., 2006), thresholded at 20 using *fslmaths*. In addition, a study specific fMRI based ROI was created by combining mean activation of both SCT and VGT for the whole study group.

6.4.5 Graphic visualization

For graphic visualization of structural MRI images, the FSL's slicer tool was used. For visualization of the results of analysis and ROIs, the FSL's slicer tool and the MRIcroGL V1.2.20201102 tool (https://www.nitrc.org/projects/mricrog) (Rorden and Brett, 2000) with the python script were employed.

6.5 Statistical analysis

Statistical evaluation was performed with the following programs: statistical package SAS (SAS Institute INC, Cary, NC), version 9.1 (substudy II) and version 9.4 (substudies I and III), R version 3.6.2 (substudies I and III) and R Studio version 1.3.1093 (substudies I and III).

The normality of data was evaluated using the Kolmogorov-Smirnov criterion (substudy II) and Shapiro-Wilk test (substudies I and III).

Continuous values are presented as means with the 95% confidence interval (CI) (substudies I–III), SD (substudies I and III) or median values with the interquartile range (substudies I and III). Qualitative variables are presented as absolute and relative frequencies (substudies I–III).

To compare proportions, the Chi-square test and Fisher's exact test (when the expected values were <5) were used.

For group comparisons between the AIS, PVI and control groups, analysis of variance or Kruskal-Wallis test was used for continuous variables (substudies I and III). Multiple comparisons were carried out if a significant difference across the groups was noted. For pairwise comparisons, Student's t test (substudy I), analysis of variance model with Tukey's post hoc test (substudy III) or Wilcoxon-Mann-Whitney test (Substudies I–III) was used. All p values are two-sided. Alpha level for determining significance was $p = 0.05$ (substudies II and III).

Correction for multiple comparisons was applied to control FDR as explained by Benjamini and Hochberg in 1995 (Benjamini and Hochberg, 1995). Multiple significance levels of FDR from 0.05 to 0.1 were used to eliminate the risk of missing potentially important findings by setting the proportion of false negatives too high.

To assess associations between imaging and clinical outcome, Pearson's correlation or nonparametric Spearman's correlation coefficient was used in substudies I and III. Additionally, simple linear regression or robust regression linear analysis was employed to estimate associations between LI and cognitive outcome (substudy III).

6.6 Ethical approval and declarations

The study was approved by the Research Ethics Committee of the University of Tartu (protocol number 170/T-17 (from April 28, 2008); 254/M-25 (from December 21, 2015), 267/M-17 (from February 20, 2017); 294/M-18 (from June 17, 2019). All procedures performed within the studies involving human participants were in accordance with the ethical standards of the University of Tartu and with the 1964 Helsinki declaration and its later amendments, or with comparable ethical standards. Written informed consent was given by all individual participants older than seven years and by their parents.

7 RESULTS

7.1 Motor outcome and volumetric changes (Substudy I)

7.1.1 Demographics

The final study group consisted of 34 children with perinatal stroke and 42 healthy controls, with their demographic data shown in Table 1. The perinatal stroke group consisted of 16 children with AIS (8 boys) with an age range of 4.86–17.15 years at the time of MRI study and 18 children with presumed PVI (7 boys) with an age range of 6.78–15.86 years at the time of MRI study. The control group consisted of age and gender matched 42 children (24 boys), age range 8.07–17.86 years at the time of MRI study. There were no differences in gender, mean age at outcome evaluation or the time of MRI investigation between the control, AIS and PVI groups. The median of the PSOM values, 2.0 for both AIS and PVI groups, was not statistically different ($p = 0.7$) between the groups.

Table 1. Demographics of the study groups in substudy I.

Demographics	AIS (n = 16)	PVI (n = 18)	Control (n = 42)	p-value
Gender Boys/girls	8/8	7/11	24/18	0.429 #
Side of stroke (MRI) left; n (%)	12 (75%)	12 (66.7%)		0.715 *
Age (years) at MRI; mean (95% CI), [range]	11.39 (9.47–13.31) [4.86–17.15]	10.38 (9.04–11.73) [6.78–15.86]	11.59 (10.79–12.39) [8.07–17.86]	0.253 $
AIS classification				
Proximal M1 of the MCA; n (%)	4 (25%)			
Distal M1 of the MCA; n (%)	5 (31.25%)			
Anterior trunk; n (%)	3 (18.75%)			
Posterior trunk; n (%)	4 (25%)			
Age (years) at AHA; mean (95% CI), [range]	8.47 (6.93–10.00) [4.6–13.2]	7.45 (6.18–8.73) [3.5–12.7]		0.208 &
AHA unit; mean (95% CI)	58.56 (48.63–68.50)	64.22 (58.1–70.27)		0.297 §
Right sensorimotor deficit (according to PSOM)				0.272 *
Normal; n (%)	1 (6.3%)	0 (0%)		
Mild; n (%)	4 (25%)	1 (5.6%)		
Moderate; n (%)	4 (25%)	8 (44.4%)		
Severe; n (%)	7 (43.8%)	9 (50.0%)		
PSOM; median (IQR), [range]	2.0 (1.5–2.75) [0.5–7.0]	2.0 (1.0–3.0) [0.5–5.0]		0.713 &
MACS				0.348 *
MACS level I; n (%)	6 (37.5%)	5 (27.8%)		0.545 ‡ #
MACS level II; n(%)	3 (18.8%)	8 (44.4%)		
MACS level III; n (%)	6 (37.5%)	5 (27.8%)		
MACS level IV; n (%)	1 (6.3%)	0 (0%)		

Chi-square; * Fisher's exact; $ analysis of variance; & Wilcoxon-Mann-Whitney; § t-test; ‡ Reference group: patients with MACS levels II, III or IV. Abbreviations: AHA, Assisting Hand Assessment; AIS, arterial ischemic stroke; CI, confidence interval; IQR, interquartile range; MACS, Manual Ability Classification System; MCA, middle cerebral artery; MRI, magnetic resonance imaging; n, number of patients; PSOM, Pediatric Stroke Outcome Measure; PVI, periventricular venous infarction;

7.1.2 Hand function according to Assisting Hand Assessment and Manual Ability Classification System

Unilateral spastic hemiparesis was present in 69% of the patients with AIS (11/16) and in 97% (17/18) of the patients with PVI, which was not statistically different between the two groups ($p = 0.27$) (Table 1). Affected hand function according to AHA was poor in both AIS and PVI groups. The mean AHA units were not statistically different in the PVI group compared to the AIS group (64.22 and 58.56, respectively; $p = 0.3$) (Table 1).

Manual ability according to MACS did not show a significant difference ($p = 0.348$) between AIS and PVI (Table 1). Minor difficulties in handling age-appropriate objects (MACS level I) were found in 37% of the patients with AIS and in 28% of the patients with PVI ($p = 0.545$), while the rest of the patients were classified to have more severely impaired manual ability (MACS levels II–IV) (Table 1).

7.1.3 Voxel based morphometry

Differences in gray matter localization between the PVI, AIS and control groups, calculated by using voxel based morphometry for the most informative slices, are presented in Figure 3.

The AIS group had less gray matter, compared to the control group, in the ipsilesional hemisphere, specifically in the cortex corresponding to the lesion and in the nucleus caudatus, putamen, globus pallidus, thalamus, hippocampus and amygdala (Figure 3a). The AIS group had more gray matter, compared to the control group, in the contralesional hemisphere: cerebral cortex, thalamus and hippocampus (Figure 3b).

Compared to the control group, the gray matter volume was smaller in the PVI group in the ipsilesional nucleus caudatus, in ipsi- and contralesional thalami (Figure 3c) and in the ipsilesional motor cortex (Figure 3d). No contra-lesional subcortical or cortical region, except for the contralesional thalamus, showed significantly smaller gray matter volume in children with PVI compared to controls. Part of the contralesional occipital lobe, but no subcortical region, showed significantly more gray matter in children with PVI compared to controls.

Children with AIS had less gray matter compared to children with PVI in the subcortical ipsilateral structures (putamen, hippocampus, amygdala). No region displayed significantly more gray matter in AIS compared to PVI.

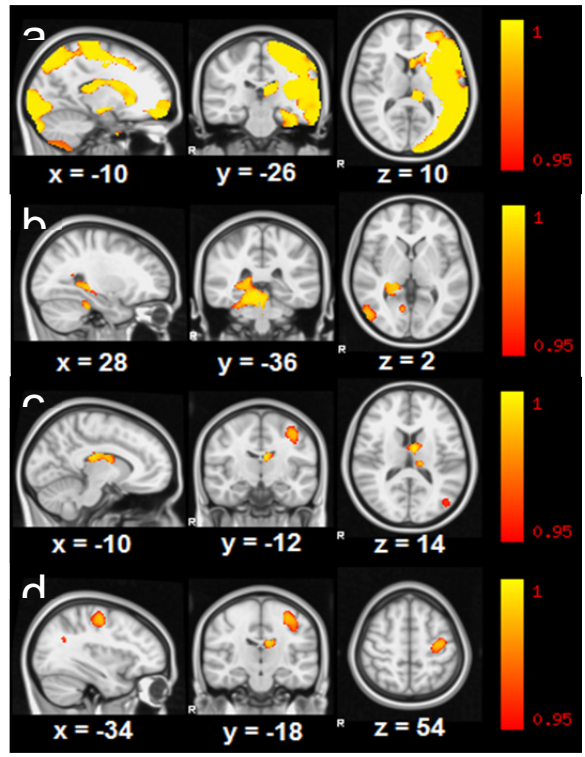

Figure 3. The results of FSLVBM (voxel based morphometry) (substudy I) revealing areas of significant differences in gray matter volume between the study groups: (a) Control > AIS; (b) AIS > Control; (c, d) Control > PVI. All maps show FWE (family-wise error) and tfce (threshold-free cluster enhancement) corrected t-stats (1-p) > 0.95 with MNI (Montreal Neurological Institute) coordinates indicated. Differences in the thalamus and nucleus caudatus for both PVI and AIS (a, c, d); cortex and putamen in (a) and motor cortex (c) are shown.

7.1.4 Volumetric analysis by segmentation

The normalized mean and median volumes (mm^3) of the subcortical structures are shown in Table 2. Although no significant difference in the mean age of the children between the study groups was found (Table 1), the scaling factors were significantly higher for AIS ($p = 0.005$) and PVI ($p = 0.0001$), compared to the control group, indicating smaller global brain size in children with perinatal stroke compared to the control group (Table 2); however, the scaling factors did not differ between PVI and AIS.

Table 2. Volumes of normalized subcortical brain structures with between-group comparisons.

Structure normalized [mm3]	Control n = 42	AIS n = 16	PVI n = 18	Control-AIS	Control-PVI	AIS-PVI
Scaling factor for head size; mean (95% CI)	1.38 (1.34–1.41)	1.47 (1.40–1.55)	1.51 (1.45–1.57)	**0.005**	**<0.0001**	0.369
Range of scaling factor	1.15–1.62	1.26–1.69	1.32–1.69			
Thalamus, ipsilesional; median (IQR)*	11548 (10930–11948)	9154 (5487–10017)	9177 (8355–10069)	**<0.0001**	**<0.0001**	0.617
Thalamus, contralesional; mean (95% CI)	11009 (10787–11230)	10312 (9872–10752)	10676 (10257–11095)	**0.003**	0.128	0.171
Caudate nucleus, ipsilesional; mean (95% CI)	5694 (5499–5889)	4905 (4083–5727)	4768 (4283–5252)	0.064	**0.001**	0.756
Caudate nucleus, contralesional; mean (95% CI)	5861 (5684–6037)	5915 (5433–6397)	5770 (5416–6123)	0.787	0.638	0.537
Putamen, ipsilesional; median (IQR)*	7355 (6979–7911)	6332 (4198–7756)	7103 (5981–7496)	0.048	0.083	0.523
Putamen, contralesional; mean (95% CI)	7326 (7130–7522)	7851 (7355–8348)	7217 (6889–7544)	0.050	0.545	0.027
Globus pallidus, ipsilesional; median (IQR)*	2443 (2331–2535)	2138 (1646–2346)	2231 (2099–2356)	**<0.001**	**<0.001**	0.277
Globus pallidus, contralesional; mean (95% CI)	2501 (2443–2558)	2465 (2318–2612)	2540 (2397–2683)	0.633	0.600	0.444
Hippocampus, ipsilesional; median (IQR)*	5265 (4994–5451)	4422 (3875–5049)	4862 (4540–5283)	**0.004**	**0.031**	0.125
Hippocampus, contralesional; mean (95% CI)	5293 (5141–5445)	5379 (5044–5714)	5210 (4933–5486)	0.586	0.583	0.361
Amygdala, ipsilesional; mean (95% CI)	1610 (1504–1716)	1421 (1212–1629)	1537 (1377–1697)	0.068	0.458	0.334

Structure normalized [mm3]	Control n = 42	AIS n = 16	PVI n = 18	Control-AIS	Control-PVI	AIS-PVI
Amygdala, contralesional; mean (95% CI)	1540 (1454–1626)	1554 (1365–1743)	1562 (1416–1709)	0.877	0.796	0.936
Nucleus accumbens, ipsilesional; mean (95% CI)	756 (711–801)	524 (416–633)	701 (601–802)	**<0.0001**	0.268	**0.004**
Nucleus accumbens, contralesional; median (IQR)*	593 (534–692)	631 (477–705)	611 (523–686)	0.924	0.790	0.904

Subcortical brain structures segmented using the FSL's FIRST tool and the volumes normalized by the FSL's SIENAX tool's volumetric scaling factor in mm3. Abbreviations: AIS, arterial ischemic stroke; CI, confidence interval; IQR, interquartile range; PVI, periventricular venous infarction. Between-groups comparison, using analysis of variance or Kruskal-Wallis test (in absence of normal distribution denoted with *). After FDR correction, using level 0.05, the cutoff p-value for significance of a single comparison was 0.033.

Both ipsi- and contralesional thalamus, ipsilesional hippocampus, globus pallidus and nucleus accumbens had a significantly smaller volume in the AIS group compared to healthy controls (Table 2).

A significantly smaller volume of the ipsilesional caudate nucleus, globus pallidus, thalamus and hippocampus was found in the PVI group compared to healthy controls.The contralesional subcortical structures displayed no significant difference in the PVI group compared to the control group (Table 2).

7.1.5 Correlations between volumetric analysis by segmentation and affected hand function according to Assisting Hand Assessment

Pearson's and Spearman's rank correlations between normalized subcortical volumes and AHA units are presented in Table 3. Figure 4 illustrates relationship between affected hand function and normalized subcortical volumes using a linear regression line for the structures for which the relationship was significant and for which the use of a linear model was valid.

In children with AIS smaller head size (indicated by higher subcortical volumetric scaling factor) correlated with worse affected hand motor function evaluated by AHA (Table 3, Figure 4a). The finding was not present in children with PVI.

In the group of children with AIS, larger size of the thalamus, globus pallidum, putamen and hippocampus in the lesion side, as well as of ipsi- and contralesional amygdala was correlated with higher AHA units (better bimanual motor function) (Figure 4e, f, d, I; Table 3). Larger size of the contralesional putamen and hippocampus in children with AIS was correlated with lower AHA units (worse bimanual motor function) (Figure 4g, h; Table 3).

In children with PVI, larger volume of the ipsilesional thalamus, nucleus caudatus and contralesional thalamus was correlated with better bimanual motor outcome (higher AHA units) (Figure 4b, c; Table 3).

Table 3. Correlation of AHA units with normalized subcortical brain structures.

Structure	AIS n = 16		PVI n = 18	
	r	p	r	p
Scaling factor for head size	-0.60	**0.013**	-0.30	0.231
Thalamus, ipsilesional	0.66	**0.006**	0.83 *	**<.0001**
Thalamus, contralesional	-0.11	0.680	0.57	**0.013**
Caudate nucleus, ipsilesional	0.35	0.179	0.62	**0.007**
Caudate nucleus, contralesional	-0.47	0.065	-0.01	0.958
Putamen, ipsilesional	0.55	**0.026**	0.20	0.428
Putamen, contralesional	-0.57	**0.022**	-0.25	0.310
Globus pallidus, ipsilesional	0.59	**0.016**	0.46 *	0.053
Globus pallidus, contralesional	0.09	0.748	0.09	0.709
Hippocampus, ipsilesional	0.51 *	**0.044**	0.16	0.527
Hippocampus, contralesional	-0.53	**0.033**	-0.01	0.981
Amygdala, ipsilesional	0.59 *	**0.015**	0.30	0.230
Amygdala, contralesional	0.62	**0.010**	-0.14	0.579
Nucleus accumbens, ipsilesional	0.41	0.111	0.18	0.463
Nucleus accumbens, contralesional	0.42	0.104	-0.17	0.496

Abbreviations: AIS, arterial ischemic stroke; n, number of patients; PVI, periventricular venous infarction. Volumes normalized for head size using the FSL's SIENAX tool's volumetric scaling factor. Pearson's correlation coefficient (r) is provided in case the assumptions of the linear regression model are satisfied. When these assumptions are not satisfied, Spearman's rank correlation coefficient is provided and denoted by*. After FDR correction, using level 0.08, the cutoff p-value for significance of a single comparison was 0.048 (for the AIS group) and 0.016 (for the PVI group) marked in bold.

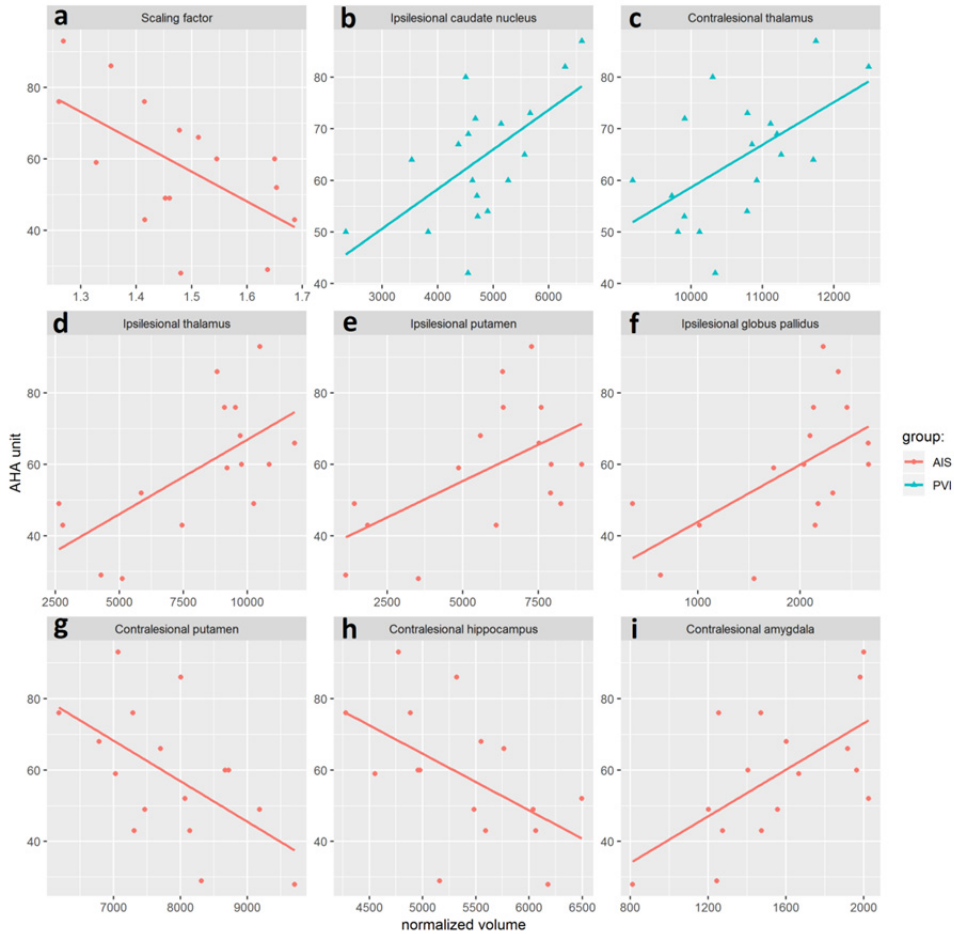

Figure 4. Statistically significant linear correlations between affected hand function, based on AHA test, and volume of the subcortical structures for children with AIS and PVI. (a) subject's head size measured by using FSL's SIENAX tool's volumetric scaling factor, (b) ipsilesional caudate nucleus, (c) contralesional thalamus, (d) ipsilesional thalamus, (e) ipsilesional putamen, (f) Ipsilesional globus pallidus, (g) contralesional putamen, (h) contralesional hippocampus, (i) contralesional amygdala.

7.1.6 Correlations between volumetric analysis by segmentation and bimanual hand function according to the Manual Ability Classification System

No significant difference in the mean normalized volumes of the subcortical structures in relation to the bimanual hand function measured for the different MACS levels was found between children with AIS and PVI (Table 4).

Table 4. Comparison of normalized subcortical brain structure volumes in mm^3 in children with AIS and PVI at different bimanual motor outcome levels of the MACS scale.

Structure normalized [mm3]	AIS n = 16				PVI n = 18			
MACS	I n = 6	II n = 3	III-IV n = 7	p-value	I n = 5	II n = 8	III-IV n = 5	p value
	mean (95% CI) or median (IQR)*	mean (95% CI) or median (IQR)*	mean (95% CI) or median (IQR)*		mean (95% CI)	mean (95% CI)	mean (95% CI)	
Thalamus, ipsilesional	9919 (8758–11080)	9945 (7865–12025)	5485 (2991–7980)	**0.003 #$**	10649 (8721–12577)	9162 (8578–9746)	6895 (4187–9603)	**0.004 #$**
Thalamus, contralesional	10110 (9643–10307)*	10237 (10227–11289)*	10291 (9587–10988)*	0.861 *	11371 (10364–12378)	10544 (9830–11258)	10190 (9709–10672)	0.062
Caudate nucleus, ipsilesional	5266 (4345–6186)	5872 (2560–9183)	4182 (2449–5915)	0.355	5416 (4205–6628)	4801 (4218–5383)	4067 (2769–5364)	0.082
Caudate nucleus, contralesional	5506 (4761–6252)	5987 (4539–7435)	6235 (5211–7258)	0.372	5664 (4678–6649)	5825 (5178–6472)	5787 (4954–6620)	0.931
Putamen, ipsilesional	6778 (5926–7629)	7242 (1967–12518)	4314 (1469–7159)	0.248	7099 (5677–8522)	6984 (6344–7624)	6505 (5156–7855)	0.590
Putamen, contralesional	7171 (6486–7856)	8140 (5749–10530)	8311 (7508–9114)	0.063	7007 (5952–8063)	7185 (6764–7605)	7476 (6569–8384)	0.550
Globus pallidus, ipsilesional	2324 (2097–2551)	2148 (974–3322)	1459 (723–2194)	0.122	2415 (2052–2778)	2236 (2107–2365)	1953 (1456–2451)	0.055

Structure normalized [mm3]	AIS n = 16				PVI n = 18			
MACS	I n = 6	II n = 3	III-IV n = 7	p-value	I n = 5	II n = 8	III-IV n = 5	p value
Globus pallidus, contralesional	2469 (2209–2730)	2606 (1657–3556)	2400 (2147–2653)	0.588	2575 (2145–3005)	2604 (2373–2834)	2403 (2082–2724)	0.478
Hippocampus, ipsilesional	4831 (4252–5410)	4911 (3044–6778)	3881 (2874–4888)	0.120	4680 (3787–5573)	4960 (4631–5288)	4619 (3518–5720)	0.604
Hippocampus, contralesional	5095 (4516–5673)	4828 (4233–5424)	5858 (5431–6286)	0.010	5140 (4560–5720)	5193 (4652–5734)	5306 (4573–6038)	0.901
Amygdala, ipsilesional	1647 (1312–1983)	1474 (1178–1770)	1203 (805–1602)	0.116	1567 (1229–1904)	1602 (1307–1898)	1402 (981–1824)	0.565
Amygdala, contralesional	1705 (1381–2030)	1679 (985–2374)	1371 (1025–1716)	0.196	1605 (1135–2074)	1531 (1264–1799)	1569 (1317–1821)	0.918
Nucleus accumbens, ipsilesional	632 (439–825)	584 (61–1108)	407 (245–568)	0.110	664 (451–877)	786 (675–897)	603 (243–963)	0.306
Nucleus accumbens, contralesional	630 (501–759)	703 (613–793)	500 (349–651)	0.093	572 (428–717)	655 (534–777)	601 (544–658)	0.458

AIS, arterial ischemic stroke; CI, confidence interval; IQR, interquartile range; MACS, Manual Ability Classification System; PVI, periventricular venous infarction; * = absence of normal distribution (Kruskal-Wallis test used instead of analysis of variance); # = mean volume difference between subjects with MACS I and III-IV; $ = mean volume difference between subjects with MACS II and III-IV; bold font = significant p-value after FDR correction (using level 0.05, the single comparison cutoff p-value for both the PVI and AIS groups is 0.0042);

The mean volumes of the ipsilesional thalamus had significant differences for the different MACS levels in both AIS and PVI groups (Table 4). However, the mean volumes of the other investigated structures showed no statistically significant difference between the different MACS levels. The subgroup of children with AIS whose bimanual hand function (MACS III–IV) was more severely affected had a smaller mean volume of the ipsilesional thalamus compared to children with less severe hemiplegia (MACS level I ($p = 0.002$) and MACS level II ($p = 0.006$)). Also in children with PVI, a significantly smaller mean volume of the ipsilesional thalamus was associated with severe hemiplegia (MACS levels III–IV) compared to those with less severely affected bimanual hand function (MACS level I ($p = 0.001$) and MACS level II ($p = 0.016$)).

7.2 Resting state functional connectivity and cognitive impairment in children with perinatal stroke (Substudy II)

7.2.1 Demographics

The final study group consisted of 17 children with perinatal stroke and 19 controls. The demographic and neuroimaging data of the children with perinatal stroke is shown in Table 5. The perinatal stroke group comprised 10 children with presumed PVI (3 boys) with an age range of 7.6–15.9 years at the time of MRI study and 7 children with neonatal or presumed perinatal AIS (5 boys), with an age range of 10.4–17.4 years at the time of MRI study. The control group consisted of age and gender matched 19 children (9 boys) with an age range of 8.1–17.9 years at the time of MRI study. There was no significant difference in age or gender between the study groups.

Table 5. Demographic and neuroimaging data for children with perinatal stroke in substudy II

Patients number	Gender	Gestational age at birth	Presumed or neonatal stroke	Type of stroke	Age at the time of resting-state functional MRI	Lesion location (left)	Lesion size 1–5
(1)	M	40	Presumed	PVI	15.9 years	F	2
(2)	F	40	Presumed	PVI	7.6 years	F	2
(3)	F	42	Presumed	PVI	10.6 years	F	2
(4)	M	40	Presumed	PVI	13.4 years	F	2
(5)	F	38	Presumed	PVI	14.6 years	Th-F	2
(6)	F	36	Presumed	PVI	14.6 years	P	1
(7)	F	38	Presumed	PVI	9.7 years	BG-Th-F-P	4
(8)	M	37	Presumed	PVI	10.8 years	BG-Th-F-P	4
(9)	F	34	Presumed	PVI	12.7 years	BG-Th-F-P	4
(10)	F	40	Presumed	PVI	8.6 years	F-P	4
(11)	M	42	Neonatal	AIS/PT	10.5 years	Th-P	3
(12)	F	38	Presumed	AIS/AT	15.3 years	F	3
(13)	M	40	Neonatal	AIS/DMI	10.7 years	Th-F-P	5
(14)	M	41	Neonatal	AIS/PT	10.5 years	F-T	5
(15)	M	39	Presumed	AIS/PMI	14.1 years	BG-Th-F-P	5
(16)	M	40	Presumed	AIS/PMI	17.4 years	BG-Th-F-P-T	5
(17)	F	39	Presumed	AIS/DMI	16.3 years	Th-F-P-T	5

Type of stroke: PVI: periventricular venous infarction; AIS: arterial ischemic stroke; PT: posterior trunk of the middle cerebral artery (MCA); AT: anterior trunk of the MCA; PMI: proximal MCA; DMI: distal MCA; Gender: M: male, F: female; Lesion location: BG: basal ganglion; Th: thalamus; F: frontal cortex; P: parietal cortex; T: temporal cortex.; Grading system for lesion size: (1) ventricular dilatation or brain atrophy; (2) focal periventricular damage involving one lobe only; (3) focal cortical damage involving one lobe only; (4) focal periventricular damage involving multiple lobes; (5) focal cortical damage involving multiple lobes.

7.2.2. Neurodevelopmental outcome

The individual clinical findings and motor and cognitive outcomes for subjects with perinatal stroke for the resting state fMRI substudy are provided in Table 6. All children with perinatal stroke had abnormal total PSOM scores, however, children with AIS had higher total PSOM scores compared to children with PVI ($p = 0.0486$). All children with perinatal stroke had mild to severe sensorimotor deficit. The proportion of moderate to severe hemiparesis (unilateral sensorimotor deficit with impaired or missing function) was 4/7 (57%) for children with AIS and 8/10 (80%) for children with PVI, with no statistical difference between the groups ($p = 0.59$).

According to PSOM, most children with AIS (5/7, 71%) and only one child with PVI (1/10, 10%) had cognitive deficit, which was a significant difference between the groups ($p = 0.035$).

The majority of the children with AIS (5/7) had epilepsy and received antiepileptic medication; all children with AIS involving several lobes, but none of the 10 children with PVI had epilepsy (p = 0.0034).

Table 6. Clinical data and data of cognitive function for children with perinatal stroke in substudy II.

Number of Patient	Type of stroke	Severity of right hemi-paresis mild/ mode-rate/ severe	PSOM	Cognitive dysfunction no/mild/ moderate/ severe	Epilpesy Yes/no	FCI score	MPI score	NVI score
(1)	PVI	Mild	0.5	No	No	111	108	127
(2)	PVI	Severe	2.5	No	No	103	97	98
(3)	PVI	Moderate	1.5	No	No	88	86	88
(4)	PVI	Severe	5	Mild	No	73	75	69
(5)	PVI	Moderate	1.5	No	No	104	104	108
(6)	PVI	Moderate	1.5	No	No	109	119	144
(7)	PVI	Moderate	1	No	No	111	97	105
(8)	PVI	Moderate	2	No	No	95	97	100
(9)	PVI	Mild	1	No	No	99	101	113
(10)	PVI	Moderate	1	No	No	99	93	101
(11)	AIS/PT	Mild	2	No	Yes	96	95	102
(12)	AIS/AT	Moderate	1	No	No	89	89	94
(13)	AIS/DMI	Moderate	3.5	Mild	No	79	78	80
(14)	AIS/PT	Mild	3	Mild	Yes	84	80	82
(15)	AIS/PMI	Severe	8	Severe	Yes	53	54	59
(16)	AIS/PMI	Severe	2.5	Mild	Yes	79	92	87
(17)	AIS/DMI	Severe	3	Mild	Yes	78	80	87

Type of stroke: PVI: periventricular venous infarction; AIS: arterial ischemic stroke; PT: posterior trunk of the middle cerebral artery (MCA); AT: anterior trunk of MCA; PMI: proximal MCA; DMI: distal MCA.

The cognitive outcome for the study groups according to the Kaufman Assessment Battery for Children II is shown in Figure 5. Children with AIS had lower cognitive outcome scores compared to healthy controls in all indices except for learning. In the PVI group, overall cognitive development remained roughly within a normal range. However, children with PVI had worse results, compared to the control group, in one of the general ability scores (FCI) and in two subscales (simultaneous and sequential information processing).

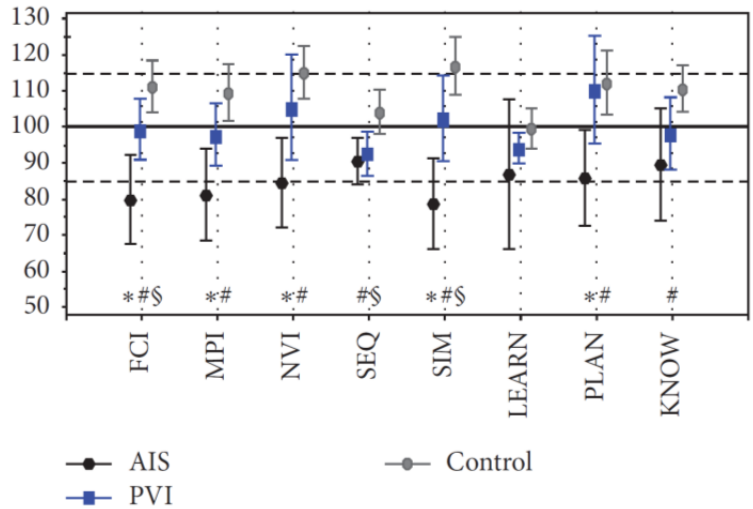

Figure 5. Mean with 95% CI Kaufman Assessment Battery for Children, Second Edition index with the subscale scores for children with periventricular venous infarction (PVI), arterial ischemic stroke (AIS), and controls. The FCI stands for Global Fluid-Crystallized Index (includes all subscales); MPI stands for Mental Processing Index (excludes acquired knowledge); NVI stands for Nonverbal Index; SEQ stands for sequential processing index; SIM stands for simultaneous information processing index; LEARN stands for learning index; PLAN stands for planning index; KNOW stands for knowledge index. Standard mean value for the battery is 100 (SD = 85–115). $*p < 0.05$ AIS versus PVI. $\#p < 0.01$ AIS versus control. $\S p < 0.05$ PVI versus control.

Children with AIS showed significantly worse cognitive outcomes compared to children with PVI according to the Kaufman Assessment Battery for Children II (Figure 5) in all general indices: FCI (mean 79.7 versus 99.2, $p = 0.013$), MPI (mean 81.1 versus 97.7, $p = 0.017$), and NVI (mean 84.4 versus 105.3, $p = 0.022$). Children with AIS had worse cognitive outcome compared to children with PVI in the subscale scores of simultaneous information processing (mean 78.6 versus 102.3; $p = 0.015$) and planning ability (mean 85.7 versus 110.2; $p = 0.017$).

7.2.2. Resting state functional connectivity.

Out of the 30 resting state networks calculated with probabilistic ICA, 13 functionally relevant networks were found, shown in Figure 6. These 13 networks were stable across the participants in the AIS, PVI and control groups. The identified functionally relevant resting state networks have been described previously for children (de Bie et al., 2012; Wylie et al., 2014). The rest of the networks corresponded to physiological noise and motion components. The default mode network in our analysis was divided into two parts, anterior and posterior, which is expected, as the functional connectivity of the default mode network increases with maturation (Sherman et al., 2014).

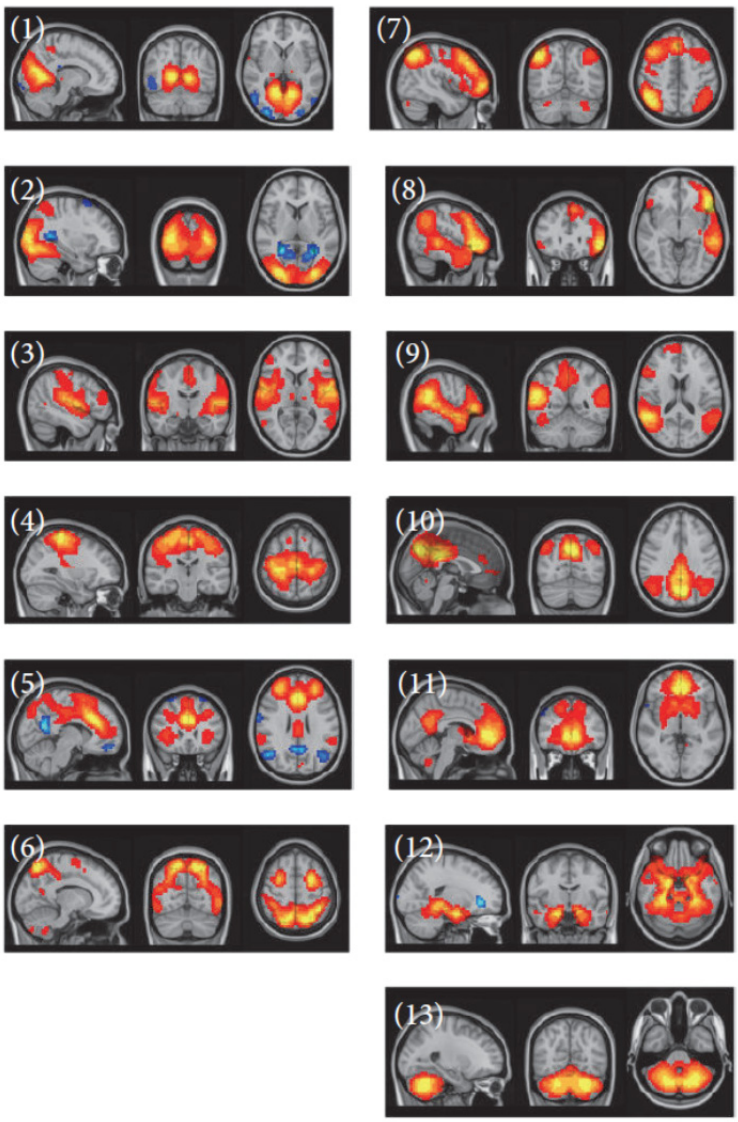

Figure 6. Resting-state networks estimated using independent component analysis in substudy II. The images are displayed using the *z* statistics of the concatenated dataset for the controls and stroke patients, decomposed into the independent network components: primary visual cortex (1), lateral visual cortex (2), auditory cortex (3), sensory-motor cortex (4) network associated with salience processing (5), task positive network involved in higher-order cognition and attention (6), networks implicated in working memory and cognitive attentional processes, as the right lateral network (7), and the left lateral frontoparietal network (8), ventral stream, ventral attention system (9), posterior component of the default mode network in the precuneus and parietal regions (10), anterior component of the default mode network in the frontal pole and precuneus (11), medial temporal/ hippocampus amygdala complex (12), and the cerebellar network (13).

7.2.3. Differences in functional connectivity between the stroke and control groups

The identified 13 resting state networks were included in group-level analysis to find group mean activation and differences between the AIS, PVI and control groups.

After FDR correction ($q \leq 0.1$), a significant groupwise activation increase was found for the AIS group vs control group ($p < 0.01$) in the posterior component of the default mode network in the left periventricular area (Figure 7). In the other networks, groupwise differences were not significant after FDR correction.

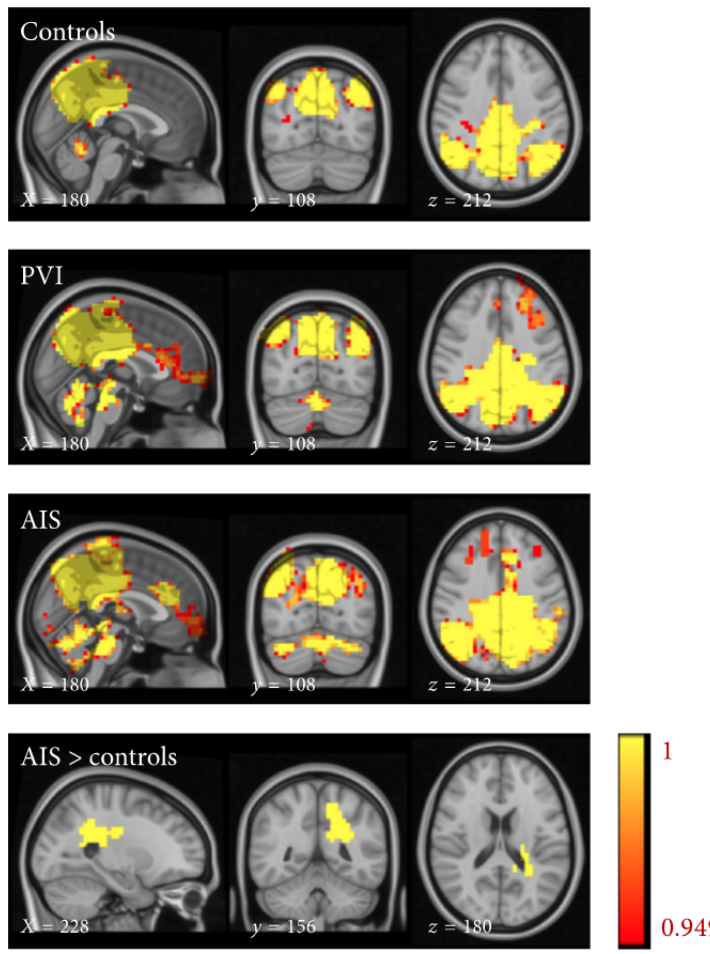

Figure 7. Connectivity maps of the posterior precuneus part of the default mode network for children of the control group, for children with periventricular venous infarction (PVI) and arterial ischemic stroke (AIS) and regions with increased activation of the default mode network in children with AIS compared to controls (FDR 0.1). Color map of (1-p) from 0.949 (red) to 1 (yellow).

7.3 Language outcome and task based functional magnetic resonance imaging (Substudy III)

7.3.1 Demographics

The final study group consisted of 21 children with perinatal stroke and 30 healthy controls, all of whom had passed at least one successful language task during fMRI scan. The final study group's demographics and the data of clinical outcome and language lateralization are provided in Table 7. The perinatal stroke group consisted of 9 children with left-side unilateral AIS (6 boys) with an age range of 9.9 to 17.3 years and 12 children with left-side unilateral PVI (6 boys) with an age range of 7.3 to 15.9 years. The control group consisted of 30 age and gender matched children (17 boys), age range 8.1 to 18.7 years. Mean age at the time of cognitive evaluation and performance of MRI was not different between the study groups (Table 7).

The axial slices of T1 weighted images of lesions for all perinatal stroke subjects are shown in Figure 8. The images are grouped according to language activation organization during VGT. Subjects without successful VGT are marked as not available (NA) in Figure 8.

Table 7. Final study group's demographics and language activation lateralization in substudy III

	AIS (n = 9)	PVI (n = 12)	Control (n = 30)	P value
Gender (Male/Female)	6/3	6/6	17/13	0.75
Gestational age in weeks, Mean (95%CI) [range]	40 (39.1–40.9) [38 to 42]	38.9 (37.8–40.1) [36 to 41]	NA	0.24
Time of diagnosis of perinatal stroke: Neonatal (<28 days)/Presumed (>28 days)	6/3	0/12	NA	0.002
Vascular type of AIS: AT	1	NA	NA	
PT	2	NA	NA	
PMI	3	NA	NA	
DMI	3	NA	NA	
Lesion in Broca area, n	4	0	NA	
Lesion in Wernicke area, n	4	0	NA	
PSOM score, median (IQR), [range]	1.5 (1.0–2.5) [0.5 to 7.0]	2.0 (1.3–2.5) [0.0 to 4.5]	NA	0.67
Moderate to severe hemiparesis, n (%)	6 (67%)	11 (92%)	NA	0.27
Epilepsy, n (%)	5 (56%)	0 (0%)	NA	0.011
Use of epilepsy medication, n	5	NA	NA	
Age in years during MRI, mean (95% CI) [range]	13.4 (11.1–15.7) [9.9 to 17.3]	11.8 (10.1–13.6) [7.3 to 15.9]	11.6 (10.6–12.6) [8.1 to 18.7]	0.22
Age in years during cognitive evaluation, Mean (95% CI) [range]	12.3 (10.1–14.4) [7.7 to 17.4]	11.1 (9.04–13.1) [7.3 to 15.9]	12.2 (11.3–13.2) [9.3 to 17.4]	0.47
Valid VGT fMRI, n	8	11	27	
LI VGT-Broca ROI, mean (SD)	NA	NA	0.54 (0.35)	
LI VGT-Broca ROI reorganized, n (%)	5 (63%)	1 (9%)	NA	
LI VGT fMRI ROI, mean (SD)	NA	NA	0.47 (0.33)	
LI VGT fMRI ROI reorganized, n (%)	5 (63%)	2 (18%)	NA	

	AIS (n = 9)	PVI (n = 12)	Control (n = 30)	P value
Valid SCT fMRI, n	7	10	25	
LI SCT Wernicke ROI, mean (SD)	NA	NA	0.24 (0.20)	
LI SCT Wernicke ROI reorganized, n (%)	6 (86%)	1 (10%)	NA	
LI SCT fMRI ROI, mean (SD)	NA	NA	0.35 (0.18)	
LI SCT fMRI ROI reorganized, n (%)	6 (86%)	3 (30%)	NA	

Data given as follows: Study group: AIS arterial ischemic stroke, PVI periventricular venous infarction; Vascular stroke type: Proximal M1 infarction (PMI), Distal M1 infarction (DMI), Anterior trunk (AT), Posterior trunk (PT); Inter-quartile range (IQR), lateralization index (LI) during verb generation (VGT) and sentence comprehension tasks (SCT) in the Broca, Wernicke and fMRI regions of interest (ROI). Lateralization of activation is classified as reorganized when it differs more than 2 SD from control group's mean LI. CI stands for confidence interval, NA stands for non-available data, n stands for group size, SD stands for standard deviation.

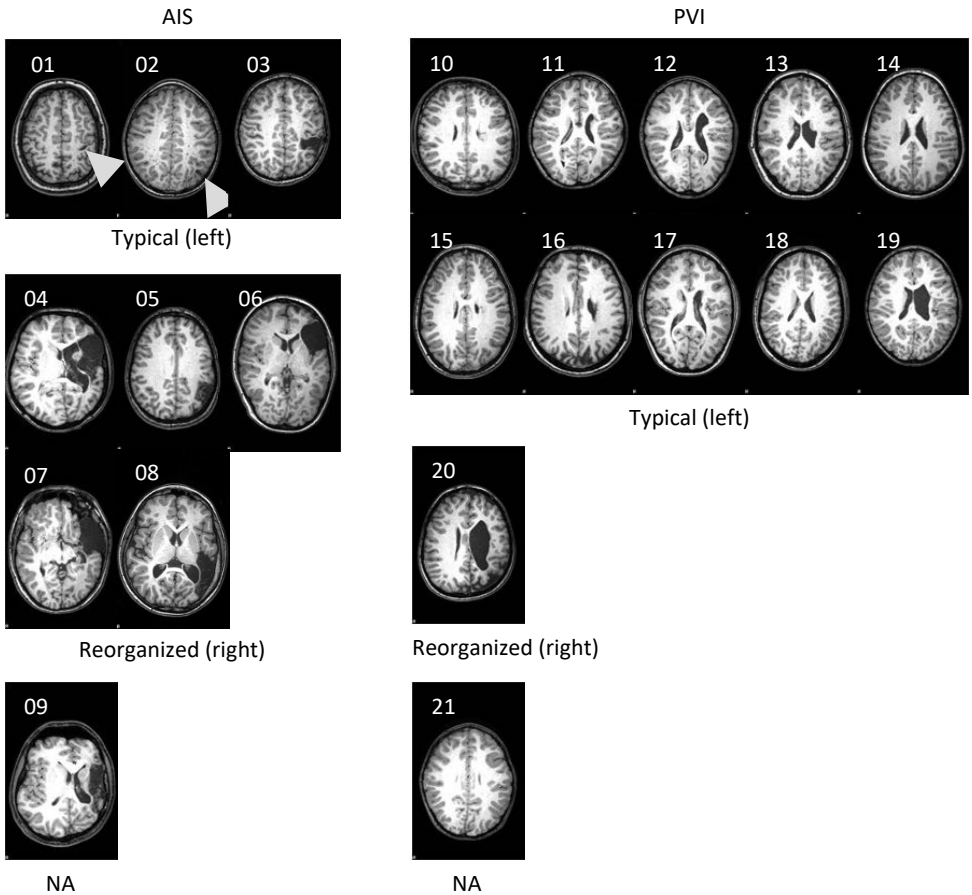

Figure 8. Representative axial slices of T1 images of all the children with AIS and PVI with subject's number in substudy III. Sorted after typical or reorganized lateralization in the Broca area during verb generation task (VGT). For two subjects VGT contained artefacts, non-available (NA).

7.3.2 Lateralization index

The ROIs used for calculations of language activation lateralization are shown in Figure 2. The red regions were used as the Broca area ROI; the green regions were used as the Wernicke area ROI in calculations of LI. The blue regions are mean activation areas for the entire study population during VGT and SCT combined to ensure the largest language task activation considered as the fMRI ROI.

Comparison of language activation LI in the AIS, PVI and control groups along with individual data points, and box diagrams during both VGT and SCT, using the Broca, Wernicke and fMRI ROIs, is shown in Figure 9.

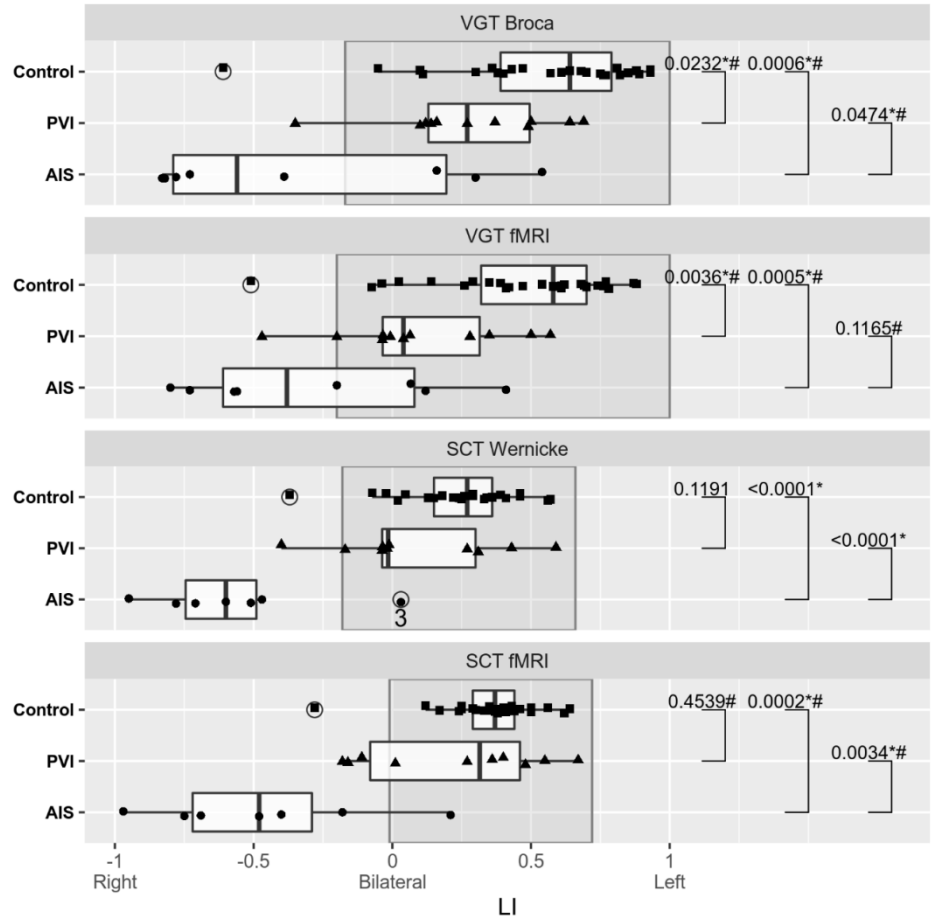

Figure 9. Individual data points and boxplots of lateralization indices (LI) for the study groups in different ROIs in substudy III. Group comparisons with FDR corrected significant p-values are denoted with *, using analysis of variance or the Mann-Whitney-Wilcoxon (denoted by #) test. Outliers are marked with black O and patient number for subjects with perinatal stroke. The typical range of LI (within ±2 SD from the control group's mean) is highlighted by the gray box.

7.3.2.1 Lateralization index during verb generation task

Children with AIS and PVI had significantly different LI compared to that of controls during VGT (Figure 9), with more rightward-lateralized LI in the AIS compared to the PVI group.

In children with large AIS lesions, LI for the Broca ROI was reorganized (median LI = -0.78 (quartile 1 = -0.82; quartile 3 = -0.73)) and significantly different (p = 0.037, Mann–Wilcoxon–Whitney test) from that of children with small AIS lesions (Figure 8 subjects 1, 2, 3) with median LI (median LI = 0.30 (quartile 1 = 0.16; quartile 3 = 0.54)).

Using a larger fMRI ROI during VGT, in the case of small lesions around the central sulcus (Figure 8, subjects 1, 3 and 5), typical lateralization was maintained; while the remaining 5/8 subjects with AIS showed reorganized lateralization.

During VGT the LI of children with PVI was different from that of AIS and control children when the Broca ROI was used (Figure 9). Typical left-side lateralization was found in the majority of the children with PVI. Lateralization was reorganized to the right contralesional side in only one child with PVI (Figure 8, subject 20) with the largest lesion, but without visible cortical involvement. One child with PVI showed reorganized language activation to the right contralesional side when the larger fMRI ROI was used (Figure 8, subjects 12 and 20).

7.3.2.2 Lateralization index during sentence comprehension task

During SCT, LI for the AIS group was significantly different from LI for both the control and PVI groups (Figure 9). Language lateralization was reorganized to the right hemisphere during SCT in the majority (6/7) of children with AIS, when both the Wernicke and fMRI ROIs were used. However, in some subjects (Figure 8 subjects 1, 5, 11 and 20) different language lateralizations were revealed during VGT and SCT tasks.

Children with large AIS lesions (PMI and DMI type) had significantly more rightward lateralization of the language function during SCT, compared to children with small AIS lesions, both when the Wernicke ($p = 0.044$ t-test) and fMRI ($p = 0.02$ t-test) ROI were used. Mean LI (95% CI) for the AIS subgroup with small lesions was -0.220 (-3.402 - 2.963) for the Wernicke ROI and 0.015 (-2.463 - 2.493) for the fMRI ROI. Mean LI (95% CI) for the AIS subgroup with large lesions was -0.710 (-0.920 - -0.500) for the Wernicke ROI and -0.658 (-0.939 - -0.377) for the fMRI ROI.

During SCT, LI for children with PVI was similar to LI for the control group and there children showed also significantly more leftward lateralization compared to children with AIS.The majority (9/10) of the children in the PVI group had typical language lateralization and only one subject (Figure 8 subject 11) was considered to have contralesional reorganized lateralization when the Wernicke ROI was used. When a larger fMRI ROI was used, two more subjects (Figure 8 subjects 19 and 20) exhibited contralesional-reorganized lateralization.

7.3.3 Cognitive outcome

Cognitive outcome was evaluated in all subjects with perinatal stroke and in the majority of control subjects (28 of 30). The cognitive outcome boxplot with comparisons between the AIS, PVI and control groups is shown in Figure10.

Children with AIS and PVI had significantly lower cognitive (FCI) and language scores (VK, EV, RID) compared to controls, except for VK in the AIS

group and EV in the PVI group (Figure 10) after FDR correction. Except in EV sub-test, there was no significant difference between the AIS and PVI groups.

General cognitive score FCI ($p = 0.10$ using t-test) showed a trend for higher values for children with small AIS lesions (mean FCI 95 (95%CI 81–109)) compared to those with large AIS lesions (mean FCI 78 (95%CI 63–93)). Moreover, median EV was significantly lower in children with AIS and large lesions (median EV = 6.5 (quartile 1 = 5; quartile 3=7)) compared to children with small lesions (median EV = 8 (quartile 1 = 8; quartile 3 = 11)) ($p < 0.05$ Mann–Wilcoxon–Whitney test). In the AIS group large or small lesion size had no effect on VK or RID outcome.

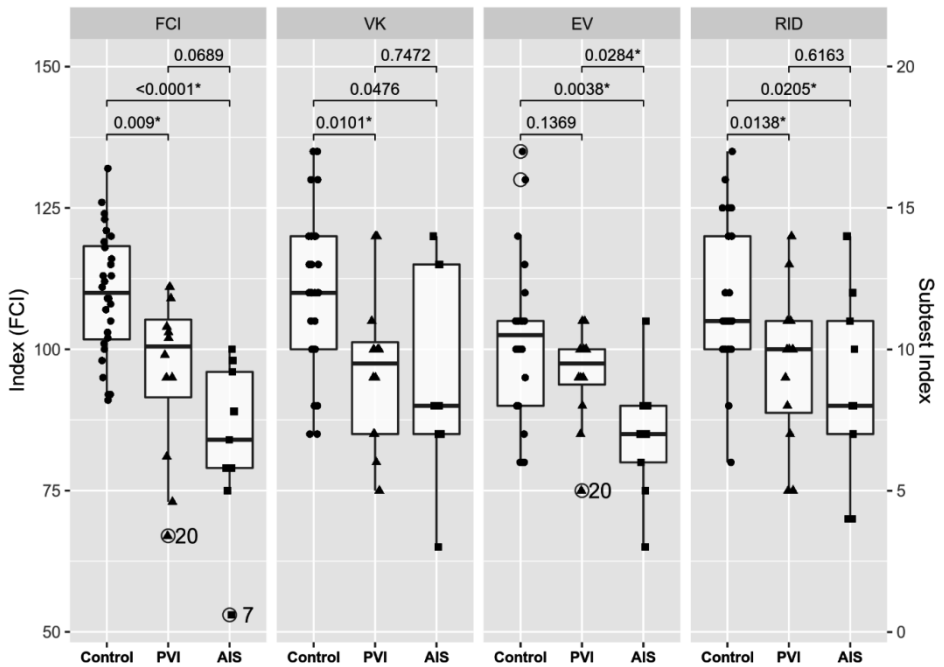

Figure 10. Cognitive outcome with individual values and boxplots of Fluid-Crystallized Index (FCI) and language subtests: expressive vocabulary test (EV), verbal knowledge test (VK) and riddles test (RID) for AIS, PVI and control groups. For group comparisons the Mann-Whitney-Wilcoxon test was used, FDR corrected significant p-values are denoted with *. Outliers are marked with black O and patient number for subjects with perinatal stroke.

7.3.4 Correlations between lateralization index and language outcome

Correlations of cognitive and language outcomes with LI in the Broca ROI during VGT are shown in Figure 11. After FDR correction, LI in the Broca ROI during VGT correlated with each of the cognitive and language outcome scores (FCI, VK, EV, RID) for the PVI group and with FCI and EV for the AIS group. There was no significant correlation between LI and cognitive performance for healthy controls.

Nor was there significant correlation of cognitive or language outcome with LI during SCT in the case of either ROIs, or with LI during VGT, when the fMRI ROI was used after FDR correction.

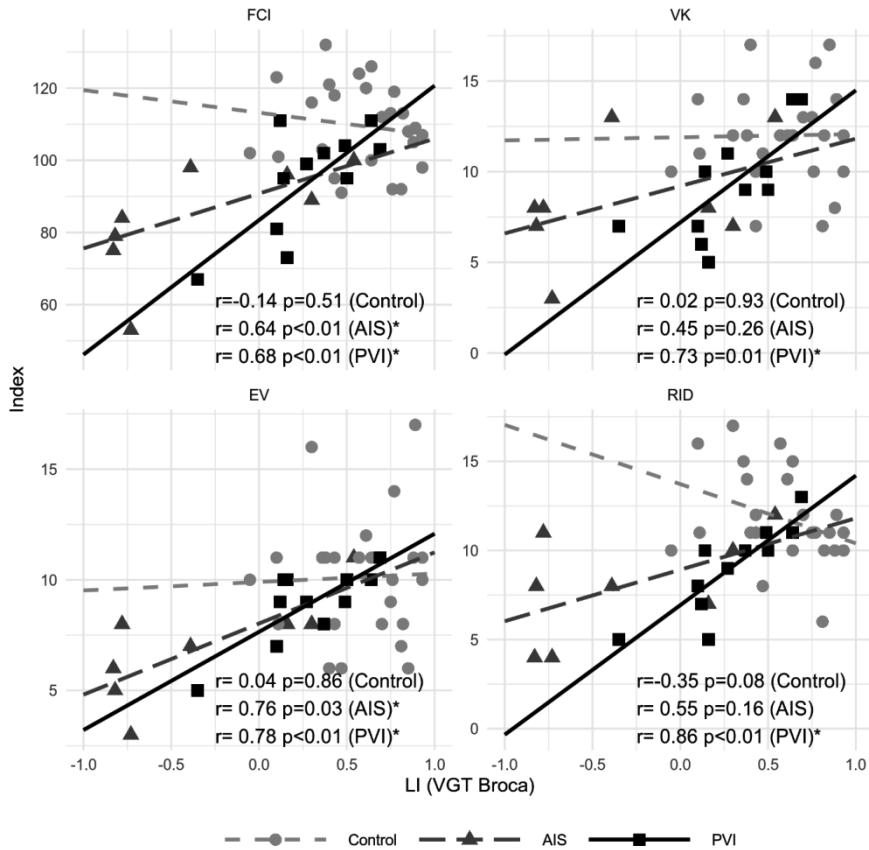

Figure 11. Scatterplots with fitted regression lines of lateralization index (LI) during verb generation test (VGT) in the Broca area against cognitive outcomes: fluid-crystallized index (FCI), expressive vocabulary test (EV), verbal knowledge test (VK) and riddles test (RID) with individual data points. The r- and p-values are provided by robust linear regression (FCI) or linear regression (VK, EV, RID). FDR corrected significant p-values are denoted with *.

Out of the cognitive and language outcomes, only VK was significantly correlated with age for children with PVI ($r = 0.70$, $p = 0.011$).

LI in the Broca ROI during VGT correlated with age of children with PVI ($r = 0.65$, $p = 0.03$), but a similar correlation did not occur for children with AIS or healthy controls.

7.3.5 Groupwise language activation analysis

The linear model used for higher level FEAT analysis provided mean activation and group comparisons during VGT for the AIS, PVI and control groups. The overall effects of confounders (gender, age, cognitive outcome (FCI) and language outcome (EV)) for the entire study population, along with mean activations, are presented in Figure 12.

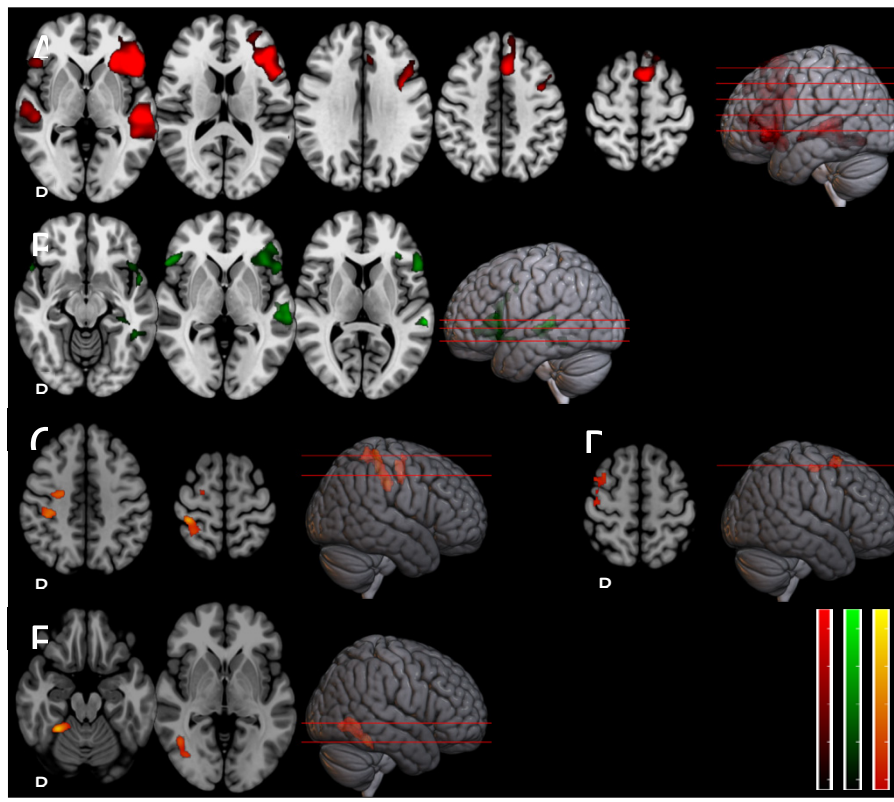

Figure 12. Verb generation task (VGT), general linear model with the confounders cognitive outcome fluid-crystallized index (FCI), expressive vocabulary test (EV), age and gender. Subfigures contain (A) mean control activation (red), (B) mean PVI activation (green), (C) controls > PVI, (D) AIS > PVI and (E) general negative effect of age. Plotted z score 3.1–5.

Figure 12 A shows how during VGT the language areas, corresponding to both the Broca and Wernicke areas, are activated in the control group. The mean activation area for the PVI group, shown in Figure 12 B, is located in the Broca area and is much smaller. There was no significant mean activation in the AIS group during VGT.

Figure 12 C shows that the networks used for language generation display more activation on the right side in the control group compared to the PVI group. Figure 12 D shows larger activation in the right hemisphere in children with AIS compared to children with PVI, which indicates that in the PVI group the language areas remain on the left, while in the AIS group they are shifted to the right.

Figure 12 E shows how activation in the cerebellum and on the right hemisphere generally decreases with age in all subjects.

The results of higher level FEAT analysis of SCT with cognitive (FCI) and language outcomes (RID) with the effects of age and gender are shown in Figure 13. Control group's mean activation (Figure 13 A) was the largest, bilateral in both hemispheres, as in VGT, and involving both the Broca and Wernicke areas. Mean activation in children with PVI (Figure 13 B) was smaller, but still bilateral, and included both the Broca and Wernicke areas. In children with AIS, mean activation (Figure 13 C) was clearly lateralized to the right hemisphere in the Wernicke area. Children with AIS showed increased activation in the right hemisphere compared to children with PVI (Figure 13 E) and controls (Figure 13 D).

During SCT, a bilateral negative effect of age was found for the entire cohort (Figure 13 F), in contrast to results from VGT, where the effect was only unilateral, in the right hemisphere (Figure 12 E). A significant positive effect of FCI for the entire cohort (Figure 13 G) was seen in the left Wernicke area during SCT, when gender was added as a confounder to the linear model.

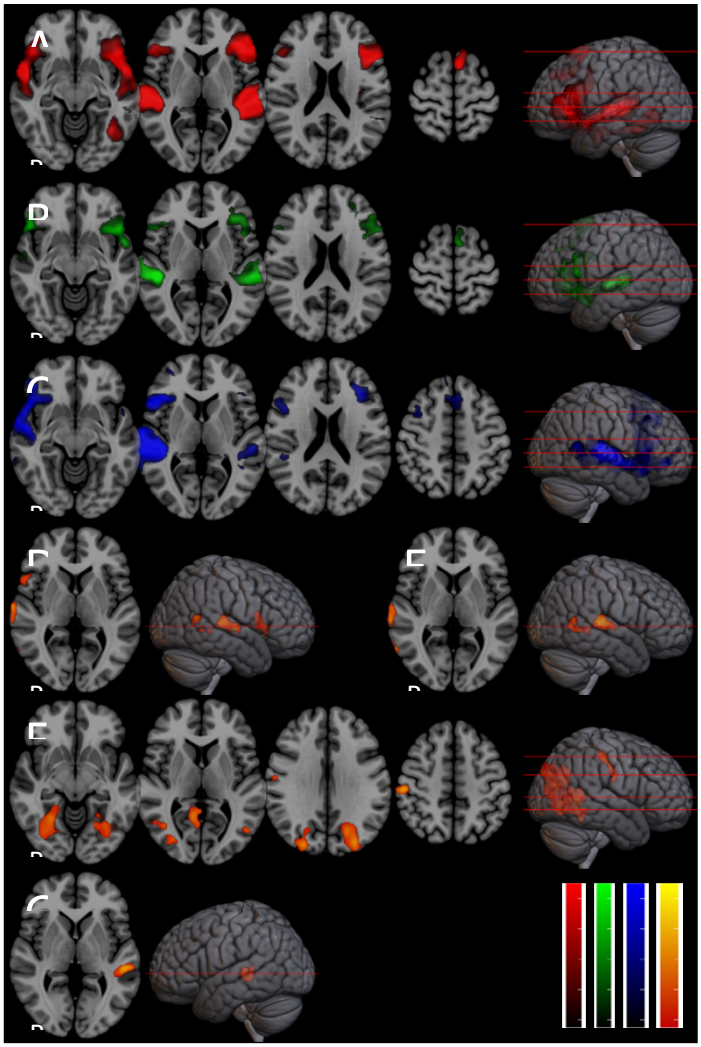

Figure 13. Sentence comprehension task (SCT), general linear model with the confounders cognitive outcome fluid-crystallized index (FCI), riddles test (RID), age and gender. Subfigures contain (A) mean control activation, (B) mean PVI activation, (C) mean AIS activation, (D) AIS > controls, (E) AIS > PVI, (F) general negative effect of age and (G) general positive effect of FCI. Plotted z score 3.1–5.

8 DISCUSSION

Perinatal stroke is a rare heterogeneous disorder, often with poor cognitive and motor outcome. There is an increasing number of various methods of therapy, rehabilitation and stimulation, the use of which would allow to improve motor outcome in children with perinatal stroke (Kirton et al., 2021). However, there is a lack of markers identifing children who will develop severe hemiparesis, poor neurocognitive or language outcome, especially if stroke is first diagnosed at the chronic stage. Therefore, it is difficult to evaluate which children develop severe disability and will benefit from early rehabilitation the most.

We used three different radiological methods to evaluate severity of brain damage, reorganization of the networks and plastic ability of the brain after perinatal stroke compared to healthy controls, i.e. resting state fMRI, volumetric analysis and task-based fMRI, and correlated the obtained findings with outcome. We found differences in motor and cognitive outcome, organization of the resting state and language networks, as well as in volumetric changes of the basal ganglia and thalamus between children with perinatal stroke and controls. Although brain plasticity and the ability to reorganize brain functionality in children with perinatal stroke help to reduce the effect of damage, these are not powerful enough. Both anatomical changes and reorganization of the functional networks of the brain may serve as prognostic markers for poor outcome in perinatal stroke patients.

We studied brain reorganization and outcome comparing children with two most frequent vascular types of perinatal stroke: children with AIS with cortical involvement and children with PVI with periventricular damage alone, and found significant differences between them. Most earlier studies have evaluated children with perinatal stroke as one group, or separately children with AIS who are the easiest to diagnose after birth. However, as the different vascular lesion types lead to different profiles of motor, cognitive and language outcomes, these children can not be evaluated as a joint study group, as concluded earlier (Kirton et al., 2008; Lõo et al., 2018). Our studies have shown that accurate radiological evaluation of perinatal stroke patients, divided into PVI and AIS according to the vascular type, is crucial, as there are significant differences in motor and cognitive outcome, as well as in brain networks and structural changes. Today, using the capabilities of modern MRI, it is easy to distinguish between PVI and AIS. Moreover, the radiological classification of lesion into PVI and AIS provides neurologists and rehabilitation specialists with information about neurocognitive and motor prognosis, and the need for rehabilitation, as shown in our study.

The population of perinatal stroke is characterized by large variation in lesion size and location, which makes it highly heterogenous. Some earlier studies have not found differences in language outcome between patients with different sizes and sites of stroke (Raja Beharelle et al., 2010). We found differences in cognitive and language outcome and in fMRI findings between

children with proximal and distal branches of MCA stroke, as well as between involvements of one or several lobes in the case of PVI damage. We have shown that, besides the vascular classification of AIS and PVI, also evaluation of lesion size and involved structures is important, as there are differences in network reorganization between proximal MCA stroke (PMI, DMI) and MCA distal cortical branch stroke (AT, PT), and between large and small periventricular damage.

8.1 Motor outcome and volumetric changes (Substudy I)

According to the study, motor outcome was poor both in children with AIS and PVI. Unilateral spastic hemiparesis was present in 69% of patients with AIS and in 97% of children with PVI, although without significant differences between our small study groups. It has been found earlier that corticospinal tract damage occurs, although in different locations, in up to 76% of children with AIS and in up to 90% of children with PVI (Husson et al., 2010; Kirton et al., 2010; Lõo et al., 2018). Hand function according to AHA and manual ability according to MACS was poor both in AIS and PVI children, but still not different in our small study groups, as found earlier (Saunders et al., 2019).

We found that, in addition to the primary stroke area, children with perinatal stroke have widespread volumetric changes in the ipsi- and contralesional subcortical structures compared to controls. Due to the high interconnectivity of the brain, focal damage, e.g perinatal stroke lesion, affects also remote brain regions. In recent years the importance of subcortical structures in predicting motor outcome following ischemic perinatal stroke has received increased attention (Craig et al., 2019a). Relative stroke volume and involvement of the basal ganglia in acute phase imaging has been shown to predict the diagnosis of cerebral palsy at 2 years of age (Wiedemann et al., 2020). However, in children with presumed perinatal stroke, neonatal imaging with diffusion-weighted imaging is not available. Our study showed that evaluation of brain damage to subcortical structures remote from primary brain damage is important in predicting motor outcome even if the child with perinatal stroke is first evaluated at the chronic stage.

The volume of subcortical structures in children with perinatal stroke was analyzed using two methods: voxel based morphometry and volumetric analysis by segmentation. Voxel based morphometry has the advantage of providing information about the entire brain; however, it requires more expertise during interpretation. Segmentation focuses on specific structures and is easier to interpret, but it requires manual work during segmentation. As the patients with perinatal stroke often have large lesions and hence deformed brains, combining complementary methods can provide results that are more reliable.

8.1.1 Differences and similarities in volume of subcortical structures between arterial ischemic stroke and periventricular venous infarction

We found that the pattern of primary stroke damage to the basal ganglia and thalamus depends on the vascular type of perinatal stroke. In AIS the size and location of damage depends on the site of thrombus and can involve the basal ganglia in proximal artery occlusion (Ilves et al., 2016; Kirton et al., 2010, 2008; Kirton and deVeber, 2009). PVI leads to lesions primarily in the periventricular area. However, in both vascular stroke types we did find volume changes in structures remote from the primary stroke area, compared to control, with different patterns of damage in AIS and PVI. Volume change in the ipsilesional thalamus was found in both AIS and PVI patients. The *nucleus caudatus* was affected the most in children with PVI and the size of the putamen, globus pallidus, amygdala and hippocampus was changed in children with AIS.

8.1.2 Correlation between volume of subcortical structures and outcome

Smaller size of the ipsilesional thalamus was correlated with poor hand function in both the AIS and PVI groups. Like in our study, volume reduction in the ipsilesional mediodorsal thalamus in children with neonatal AIS was shown to be correlated with contralesional hand motor deficit in a previous study (Dinomais et al., 2016). Correlations for other subcortical structures depended on the vascular type of perinatal stroke. Poor hand function in children with AIS was correlated with smaller size of the ipsilesional putamen, globus pallidus, hippocampus, amygdala and contralesional amygdala, but also with larger contralesional putamen and hippocampus. In children with PVI, smaller size of both thalami and the ipsilesional *nucleus caudatus* was correlated with poor hand function; this finding not been reported earlier.

Earlier data about volumetric changes in contralesional structures in children with perinatal stroke is scarce and further research is needed. A study by Craig and colleagues (Craig et al., 2019a) reported an increase in the volume of the contralesional thalamus in children with AIS versus control and PVI without significant correlation between volume and motor outcome. In contrast, in our study we found a significant reduction in the contralesional thalamus in AIS versus control, but not in PVI versus control. However, the voxel based morphometry analysis used by us, which distinguishes between changes in different locations of the same structure, showed an increased gray matter volume in part of the contralesional thalamus in children with AIS compared to control. Unlike Craig et al. (Craig et al., 2019a), we evaluated the AIS and PVI groups separately and found that smaller contralesional thalamus correlated with worse affected hand function in the PVI group, but not in the AIS group. Another reason for this discrepancy with our study may be the exclusion of the

most severely affected children in the study by Craig and colleagues (Craig et al., 2019a), which renders that study less representative for children with stroke.

According to earlier studies, the cortex is relatively well spared in children with PVI (Kirton et al., 2008; Woodward et al., 2019). We found that gray matter volume in the ipsilesional cortical motor area in children with PVI was also decreased. As the cortical motor area and the subcortical thalamus and basal ganglia are highly interconnected, reduced communication probably causes mutual reduction in size. Another explanation for cortical reduction may be changed neuron migration after damage to the germinal matrix in PVI. In an earlier resting state fMRI investigation of children with PVI, increased functional connectivity between the sensomotor cortex and thalamus was shown to be correlated with better hand position sense (Woodward et al., 2019), however, the relationship with the other basal ganglia was not evaluated.

It is very important to evaluate the size of the subcortical structures, especially the thalamus, to assess the prognostic indicators for motor outcome, if the first MRI is performed during the chronic state in children with presumed perinatal stroke. The size of the thalamus is easy to evaluate during MRI in patients with perinatal stroke and should be included in the radiological description protocol for these patients. As the pattern of damage to the other subcortical structures is different in children with PVI and AIS, the radiologist should detect the major locations of damage: in the nucleus caudatus in PVI and in other basal ganglia in AIS. Radiologic evaluation of subcortical structures could add to the understanding of developmental neuroplasticity following perinatal stroke. It is also important in predicting outcomes and the need for rehabilitation, as well as in development of individualized therapies for children with different vascular type of stroke.

8.2 Cognitive outcome and resting state functional magnetic resonance imaging (Substudy II)

8.2.1 Cognitive outcome

Our findings demonstrated differences in the cognitive function and neural network's profile of children with perinatal stroke and controls matched for age and gender. Although motor outcome based on PSOM was similar in children with AIS and PVI in the small study group, the cognitive outcome, based on the Kaufman Assessment Battery for Children II, indicated lower scores in the AIS group compared to the PVI group and healthy controls. Cognitive function was borderline normal in most of the subtests in children with AIS, while the results were significantly lower compared to the PVI and control groups. Children with PVI generally had average cognitive function, which was lower in only some subtests.

Most earlier studies evaluating cognitive outcome in perinatal stroke considered only children with AIS or combined children with all vascular stroke

types under one group. Association of cognitive abilities with stroke's vascular type can be concealed when subjects with AIS and PVI are combined in analysis (Ballantyne et al., 2008; Kolk et al., 2011; Westmacott et al., 2010). According to Ricci et al. (Ricci et al., 2008), only one-third of children with AIS have cognitive deficit during school age. It has been shown that children with AIS vs healthy controls have significantly lower scores of working memory (Boardman et al., 2005; Hajek et al., 2014), processing speed (Boardman et al., 2005), general cognitive ability, verbal functioning, inhibitory control (Hajek et al., 2014) or abstract reasoning (Westmacott et al., 2010).

In children with presumed PVI cognitive problems were less pronounced compared to patients with AIS (Lõo et al., 2018). Only one of the 10 children in our study group had borderline cognitive deficit according to PSOM. In a study by Kirton et al. (Kirton et al., 2008) 29% of children with perinatal stroke, combining both AIS and PVI, had cognitive or behavioral deficit, which was less frequent in children with PVI and more strongly correlated with cortical involvement in AIS.

Differences in the cognitive outcome of children with different vascular subtypes highlights once more the importance of evaluating outcome in children with AIS and PVI separately. Vascular classification as evaluated by MRI in children with perinatal stroke is essential also in predicting their cognitive outcome and need for rehabilitation.

8.2.2 Resting state functional magnetic resonance imaging investigations

Our study is the first to show significant global derangement of the resting state fMRI networks responsible for cognitive function in children with perinatal stroke. This study demonstrates that as the location of damage is different in AIS and PVI, also the resting state networks and cognitive outcomes are different. Increased functional connectivity only in the posterior precuneus part of the default mode network was significantly different in children with AIS, but not PVI, compared to healthy controls.

Most of the children's resting state networks, in particular basic visual, motor and sensory related networks have robust functional organization and are similar to adult network patterns (de Bie et al., 2012; Muetzel et al., 2016; Thornburgh et al., 2017). The default mode network is involved in episodic memory processes and self-referential mental representations and is deactivated during demanding cognitive tasks (Fan et al., 2021; Horowitz-Kraus et al., 2017). The default mode network and other resting state networks involved in higher-order cognitive functions had immature characteristics and fragmented patterns, as expected in children with developing brains (de Bie et al., 2012; Fan et al., 2021; Grayson and Fair, 2017; Muetzel et al., 2016). A major difference between adults and children is the fragmentation of the default mode network into several independent subsystems in children, with increased connectivity with maturation (de Bie et al., 2012; Fan et al., 2021; Horowitz-Kraus et al.,

2017). The default mode network consists of the anterior and posterior networks, with the posterior precuneus network fragment being the main and more robust hub (Muetzel et al., 2016; Thornburgh et al., 2017). Disruption of normal maturation processes in children with perinatal stroke might have a stronger impact on the functional reorganization of immature networks like the default mode network, compared to developed and robust functional networks. We demonstrated significant changes in the main precuneus posterior part of the immature default mode network in children with AIS who had also serious cognitive problems.

8.2.3 Location of stroke and resting state functional magnetic resonance imaging networks

Differences in network dysfunctions in children with AIS can be explained by more cortical involvement of damage in AIS compared to PVI, which causes primary damage in the periventricular area, sparing the cortex. The increased connectivity of the networks outside primary damage in children with AIS could serve as a compensatory mechanism produced by brain's plasticity during which a function is transferred to the undamaged brain tissue. This shift of function to the unlesioned brain tissue is analogous to results from language task-based fMRI (Bartha-Doering et al., 2019; Everts et al., 2010; François et al., 2019; Ilves et al., 2014; Lidzba et al., 2017a; Raja Beharelle et al., 2010).

Changes in the resting state networks in children with AIS could possibly serve as an underlying cause of disturbed brain cognitive functions in patients with perinatal stroke. Resting state fMRI is easily applicable even for those children with stroke who are not able to actively participate in task based fMRI. However, complicated analysis and interpretation limit its wide clinical use. In order to better understand the meaning of derangements in brain networks in patients with perinatal stroke with cognitive and behavioral problems, further investigations of resting state networks are needed. However, changes in resting state fMRI and altered connectivity (Carlson et al., 2019; Saunders et al., 2019) can promote early diagnosis, prompt treatment and rehabilitation of these children, and hence considerably improve their quality of life, as well as the quality of the life of their families.

8.3 Language outcome and task based functional magnetic resonance imaging (Substudy III)

8.3.1 Cognitive and language outcome in different vascular types of perinatal stroke

Our study shows that patients with perinatal stroke have lower general cognitive ability, along with lower specific subtest scores that measure language development compared to control, based on the Kaufman Assessment Battery for

Children II FCI scores. There were differences in language lateralization and language outcome between children with the different vascular stroke types. Language outcome was worse in children with AIS, especially those with right-side language lateralization, compared to children with PVI, as the capacity of brain plasticity is probably limited after perinatal stroke.

Children with AIS and PVI showed no difference in their VK subtest evaluating knowledge and receptive language skills, or in RID subtest evaluating comprehension and verbal reasoning abilities. However, children with AIS compared to children with PVI had lower values of EV test evaluating knowledge and expressive language. Moreover, children with large proximal artery AIS and right-side language lateralization had significantly lower language outcome indices compared to children with small stroke lesions involving only a small cortical area.

8.3.2 Language lateralization and vascular type of perinatal stroke

Language lateralization in left-side perinatal stroke depends on the vascular type of stroke. We found that in children with left-side AIS the language center was mostly lateralized to the right hemisphere and in children with PVI, to the left hemisphere. Our results are in accordance with previous studies describing reorganization of language centers to the contralesional side in children with perinatal AIS (Bartha-Doering et al., 2019; Ilves et al., 2014; Lidzba et al., 2017a, 2017b; Raja Beharelle et al., 2010; Szaflarski et al., 2014; Tillema et al., 2008). Other studies have confirmed that typical left-side lateralization of the language center is more common in children with PVI, whose lesions affect only the fronto-parietal periventricular white matter, compared to children with AIS (Brizzolara et al., 2002; Raja Beharelle et al., 2010; Staudt et al., 2001).

According to our study, the size of stroke is important in language lateralization. The language center in children with large AIS in the proximal MCA artery (DMI; PMI) displayed contralesional rightward lateralization, while small cortical lesions in the distal AT or PT of the MCA maintained typical leftward lateralization. Some previous studies have reported that small cortical lesions do not lead to atypical language lateralization (Gaillard et al., 2007; Pataraia et al., 2004), while others have not found dependence between language lateralization and lesion size or site (Raja Beharelle et al., 2010).

Analogously, children with left-side PVI without cortical damage maintained the typical leftward lateralized language center in the ipsilesional brain hemisphere. However, large periventricular injury in children with PVI can lead to contralesional right-side lateralization of the language center, as connectivity and pathways are disturbed to a larger degree (Carlson et al., 2019; Ilves et al., 2014). Previous studies have hypothesized that one of the causes of reorganization of language networks in children with PVI whose cortex is intact could be strategically located lesions affecting the articulatory motor tract (Staudt et

al., 2008, 2001). More data about children with large PVI lesions is needed to acquire conclusive knowledge.

8.3.3 Differences in language activation between verb generation task and sentence comprehension task

In healthy controls, not only isolated centers, but also the entire language network in both the Broca and Wernicke areas was activated during language generation and comprehension fMRI tasks. This is in accordance with earlier studies which suggest that language comprehension and language production recruit overlapping areas, but involve dissociable neural networks in the brain (Price, 2012).

Language comprehension and generation can be hemispherically dissociated and lateralized in the different hemispheres in some patients with perinatal stroke. This fact has been reported in previous studies of children with epilepsy (Wilke et al., 2010) and perinatal stroke (Staudt et al., 2001) and in healthy children (Lidzba et al., 2011). In our study neither of the two subjects with dissociated language lateralization had epilepsy. Earlier studies have shown that language generation is more prone to being reorganized, especially in children with PVI (Lidzba et al., 2017a). The study of Raja Beharelle and coworkers found more leftward lateralized language activation in the anterior region, which is responsible for language generation, in children with PVI compared to children with AIS. At the same time, the posterior language regions showed no difference between the different vascular types of perinatal stroke (Raja Beharelle et al., 2010).

In general, different correlations between language abilities and various fMRI tasks accentuate the requirement to employ different tasks for evaluating language function in fMRI not only in children with perinatal stroke, but also in children with other brain lesions (tumor and epilepsy cases requiring surgery).

8.3.4 Language maturation with age and lateralization

Language lateralization changes with maturation of children and continues even during school age (Everts et al., 2008; Holland et al., 2001). We found correlation between left-side lateralization and age during VGT in children with PVI, which was concurrent with improvement in language outcome in children with PVI vs AIS. Similar correlation between lateralization and age was not established in children with AIS or in healthy controls. However, there was a general trend for deactivation of language network with age across all groups in group wise analysis.

Previous studies have described more bilateral language network activation in children with perinatal stroke compared to overall left-side activation in the frontal, temporal and parietal regions during VGT in typically developing children (Szaflarski et al., 2014). The initial use of the bilateral language network with a gradual change from the bilateral to the unilateral language

network with age in children with PVI has been proposed as the brain's plasticity mechanism to compensate for functionality after childhood stroke (Everts et al., 2010). This enhanced capacity to improve language lateralization with age in children with PVI, which is not present in children with AIS, could also contribute to better cognitive and language abilities in children with PVI compared to AIS.

8.3.5 Correlation of lateralization with cognitive and language outcomes

In children with PVI better general cognitive (FCI) and language abilities were correlated with leftward lateralization in the Broca area during VGT fMRI task. In children with AIS, apart from better general cognitive abilities (FCI), only better EV was correlated with leftward language lateralization in the Broca ROI during VGT fMRI task.

Our results coincide with earlier studies in that reorganization of language function to the right hemisphere does not lead to normal language outcome in children with perinatal AIS (François et al., 2019; Raja Beharelle et al., 2010). Atypical language representation is considered to be unfavorable for language abilities in childhood stroke (Bartha-Doering et al., 2019). Neonatal brain plasticity is limited and is not always able to attain normal outcome, as suggested in a recent review (François et al., 2021).

Correlation of language activation with general cognitive outcome, as measured with FCI, indicates how important language networks are for general cognitive function. Groupwise analysis of SCT fMRI data revealed increased activation in the left Wernicke area in subjects with better general cognitive ability, which highlights similar relationship between language network and cognitive outcome. Analogous results about better language network connectivity in children with PVI and in healthy controls, associated with better language comprehension, were found in a study of resting-state fMRI (Carlson et al., 2019).

Reorganization of the language center to the right side in children with perinatal stroke is not sufficient to ensure normal level of language function. Reorganization of the language to the right hemisphere only minimizes, but does not eliminate the negative effect of large stroke in children with AIS. This indicates that brain plasticity is not powerful enough to completely eliminate damage in the neonatal brain. As suggested earlier (Lidzba et al., 2017a), language (re-)organization in children with perinatal stroke depends on the vascular type and size of stroke, which can be easily detected on routine MRI evaluation and can guide intensive rehabilitation of language function.

8.4 Limitations

8.4.1 Group size and age range

The study groups were small due to the overall low prevalence of perinatal stroke. In combination with low prevalence, heterogeneity makes standard group average-based statistics difficult to apply as reported earlier (Kirton et al., 2021). To recruit enough subjects willing and able to perform an MRI investigation without anesthesia, children with a wide age range were enrolled during a long period of time. To compensate for the heterogeneity of the patients with stroke, a large group of healthy controls was recruited.

MRI is particularly demanding for young subjects who need to remain motionless for a long time. An investigation without anesthesia necessitates smaller group size, because 1) some children with the most severe damage are not able to perform MRI, 2) some children are afraid of participation and 3) MRI investigation is long in time (approximately 45 minutes), which causes discomfort and movement artefacts. However, the study team, the study protocol, and the MRI scanner were the same throughout the study period.

MRI, but especially fMRI, often leads to aborted or canceled scans, while scheduled cognitive or motor evaluations can be performed successfully. To include more subjects, MRI was rescanned, which created a bias, as during the time that lapsed between evaluations, the child had matured and received rehabilitation. However, there were no differences in age between the study groups regarding clinical evaluation and fMRI investigation.

The consequence of the wide age range was also heterogenous head and brain size, which required extra normalization. A commonly used brain size (Kijonka et al., 2020) was not suitable for normalization, as the total brain volume of subjects with large stroke lesions is smaller than normal.

8.4.2 Lesion laterality

For homogeneity, we included only children with left-sided damage in the fMRI groups as left hemisphere lesions are more common in children with AIS (Dunbar et al., 2020). Previous studies (Ilves et al., 2014; Lidzba et al., 2017a; Staudt et al., 2002, 2001) have also recruited subjects with only unilateral left-hemisphere stroke. Laterality of perinatal stroke lesion can affect cognitive function and functional networks. A predictive value of fMRI results for perinatal stroke with right-side lesions should be established in future multicenter studies in order to include a sufficient number of patients.

8.4.3 Gender

There were no differences in the gender balance between children in the perinatal stroke and control groups. However, there may be an inherent gender dysbalance in different vascular types of perinatal stroke with a slight bias towards male predominance, especially in children with AIS (Dunbar et al.,

2020; Golomb et al., 2004; Grunt et al., 2015; Laugesaar et al., 2007; Li et al., 2017; Sorg et al., 2020). As cognitive outcome in children may depend on gender (Westmacott et al., 2009), this could have biased the PVI group and the AIS group differently.

8.4.4 Epilepsy

Epilepsy may influence cognitive function in children with perinatal stroke. In substudy III none of the children with PVI, but more than half of the children with AIS had epilepsy. However, the analysis of the fMRI data was performed without the knowledge of their status of epilepsy. Differences in epilepsy incidence between children with cortical and subcortical stroke have been reported earlier (Wagenaar et al., 2018). Most of the children with AIS (81% at the age of 8.6 years), but not with PVI, had developed epilepsy (Laugesaar et al., 2018). Furthermore, children with large stroke involving several lobes develop epilepsy more often compared to children with distal branch stroke (Laugesaar et al., 2018). A recent population based study claims that epilepsy in children with perinatal stroke might develop until adulthood (Sundelin et al., 2021). As the incidence of epilepsy is high among children with large AIS, we cannot ignore it by excluding these children from studies of the cognitive outcome of perinatal stroke.

8.4.5 Cognitive evaluation

Cognitive deficits in children with perinatal stroke may initially be concealed and may emerge with brain maturation and age (Westmacott et al., 2009). Therefore, a wide age range can create a bias in cognitive outcome, which may persist despite the use of age-adjusted norms.

8.4.6 Language

All subjects performed their fMRI tasks in their first language. However, children will have been exposed to English and other languages by teenage. The effect of exposure to multiple languages is unknown and difficult to eliminate. Rough comparison of auditory based language fMRI tasks and visual (picture based) language tests (VK and EV) was not an optimal solution.

8.4.7 Rehabilitation

Different therapies, interventions and caregivers' motivation can affect cognitive and motor outcomes, as well as language development, and can create an unknown bias in results. It was not possible to evaluate the effect and availability of rehabilitation retrospectively. However, outcome may reach a plateau at a certain age. For example, affected hand function reaches a plateau between 2.5 and 8 years of age and does not change significantly thereafter (Holmefur et al., 2010).

8.4.8 Brain distortion and image analysis

Image registration of the severely deformed brains of perinatal stroke subjects to a common standard brain is one of the major challenges of analysis (François et al., 2021). Large lesions with proximal MCA stroke in children with AIS deform the basal ganglia and cortical areas to a large extent, making it difficult to evaluate brain structures by automatic segmentation. Manual correction was used to correct errors of automatic analysis. One and the same researcher re-evaluated the data in two weeks to determine the robustness of the results during segmentation. Additionally, all subjects' data were analysed in parallel, in order to evaluate all subjects in a similar manner. The researcher evaluating fMRI and volumetry was blinded to the clinical data, and correlation between clinical data and fMRI was disclosed only after statistical analysis.

In the current study the MNI-152 standard space (Jenkinson and Smith, 2001; Jenkinson et al., 2002), which is based on adult brain images, was used for registration. Although an age specific standard template for children (Richards et al., 2016) could better reflect age specific brain maturation, the age range of the study group was large and many of the subjects were already teenagers. Due to the low resolution of fMRI data, the same stereotactic space is considered adequate even when comparing children and adults (Burgund et al., 2002). The effect of deformation of the brain on registration can be reduced by optimizing brain extraction and by including lesion maps.

8.5 Contribution to the field

Our research demonstrates that evaluation of the vascular type of perinatal stroke, i.e. AIS or PVI, size of stroke and involvement of cortical and sub-cortical structures in brain MRI are important in evaluating the prognosis of motor, cognitive and language outcomes in children with perinatal stroke. Due to heterogeneity, patients with perinatal stroke cannot be evaluated as a single group in outcome or MRI studies. Radiological classification of perinatal stroke (AIS or PVI), along with evaluation of stroke lesion size and involvement of proximal MCA or the cortical branch of MCA alone in children with AIS, or involvement of one or several lobes in children with PVI, can easily be performed by neuroradiologists. The results can be used for predicting outcome and for planning the appropriate rehabilitation. Children with large proximal stroke need early intensive multidisciplinary rehabilitation. Accurate vascular classification of stroke and evaluation of the thalamus and basal ganglia can provide pediatric neurologists and rehabilitation specialists with a better understanding about the purpose for rehabilitation, as well as about prognosis.

Prognosis and need for rehabilitation are also important for patients and their parents. Children with stroke have a high risk of permanent lifelong disability, which may lead to depression and stress for them and for their families (Bemister et al., 2015). It is essential to promote early diagnosis, treatment and

rehabilitation of children with perinatal stroke, in order to improve their quality of life, as well as the quality of life of their families, and to relieve the social and economic burden to the society.

Generalized knowledge concerning children with perinatal stroke, their rehabilitation needs and treatment outcome can be extrapolated to cases with other focal brain damages in children like trauma or tumors. Also, this knowledge allows planning interventions, predicting outcomes and optimizing rehabilitation after brain surgery in patients with other diseases and focal brain damages. Reliable fMRI data can help plan brain surgery in the case of tumors or epilepsy in children.

9 CONCLUSIONS

- Smaller volume of the ipsilesional thalamus is associated with poor affected hand function in children with both AIS and PVI. The pattern of correlation between hand function and volume of the basal ganglia varies between PVI and AIS. Radiological evaluation of the size of thalamus, basal ganglia and other subcortical structures add important information about motor outcome following perinatal stroke.
- In children with left-side perinatal stroke, resting state networks and cognitive outcome differ from those of healthy controls. Cognitive outcome and resting state networks are different among children with perinatal stroke and depend on the vascular type of stroke. Cognitive abilities are better in children with PVI compared to AIS.
- Patients with perinatal stroke have poorer language abilities compared to controls; however, there are differences in language lateralization and language outcome between children with the different vascular stroke types and size. Language reorganization to the unlesioned right hemisphere cannot ensure normal language development and is associated with worse language abilities in these patients.

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11 SUMMARY IN ESTONIAN

Aju plastilisus ja võrgustike reorganiseerumine perinataalset insulti põdenud lastel: funktsionaalne magnetresonantstomograafiline uuring

11.1 Sissejuhatus

Insult võib tekkida kogu elu jooksul, kuid selle tekke tõenäosus on eriti suur perinataalsel perioodil (Kirton and deVeber, 2013). Perinataalne insult võib lapsele põhjustada elukestva motoorse, kognitiivse, sealhulgas kõnelis- keelelise kahjustuse, ning epilepsia (Dunbar and Kirton, 2018; Hawe et al., 2020; Laugesaar et al., 2018; Lõo et al., 2018; Virani et al., 2022). Lisaks insulti põdenud lapse enda eluaegsele füüsilisele, vaimsele ja sotsiaalsele kahjule, on insuldil ka oluline majanduslik mõju nii nende laste peredele kui ka kogu ühiskonnale.

Kaks enim levinud perinataalse insuldi tüüpi on arteriaalne isheemiline insult (AII) ja periventrikulaarne venoosne insult (PVI) (Kirton et al., 2008). AII tekib suure arteri sulguse tagajärjel enamasti keskmises ajuarteris, kusjuures kortikaalne kahjustus ja subkortikaalsete struktuuride haaratus sõltub trombi asukohast (Ilves et al., 2016; Kirton et al., 2010, 2008). AII kahjustused on ühepoolsed ja paiknevad enamasti vasakus ajupoolkeras (Dunbar et al., 2020; Grunt et al., 2015). Paljud senised uuringud on kaasanud ainult AIId põdenud lapsi, kuna neid on lihtsam ära tunda esimestel elupäevadel tekkivate epileptiliste hoogude tõttu (Boardman et al., 2005; Dinomais et al., 2015; Husson et al., 2010; Mercuri, 2003; Westmacott et al., 2009; Wiedemann et al., 2020). Ajaliselt sündinud PVIga lapsel on insult tekkinud looteeas verejooksu tagajärjel germinaalmaatriksisse 24.-34. rasedusnädalal (de Vries et al., 2001; Fluss et al., 2019). Osa looteeas insulti põdenud laste seisund stabiliseerub üsasiseselt ja nad sünnivad ajalisena ning insuldile iseloomulikud sümptomid avalduvad alles peale vastsündinuperioodi (Ilves et al., 2016). PVI järgselt tekib aju külgvatsakese porentsefaalne laienemine koos periventrikulaarse valgeaine glioosiga või tsüstiliste muutustega, kusjuures ajukoor ja basaaltuumad on vähem haaratud (Kirton et al., 2008).

Enamik varasematest töödest on uurinud erineva vaskulaarse tüübiga perinataalset insulti põdenud lapsi koos ühe uuringugrupina. Magnetresonantstomograafia (MRT) abil saab aga insulti kergesti klassifitseerida vaskulaarse tüübi järgi. Kuna ajus erineva vaskulaarse kahjustuse korral on kortikaalne haaratus erinev, siis ka aju ümberorganiseerumisvõime ja plastilisus võivad erineda.

11.2 Uuringu eesmärgid

Antud töö peamiseks eesmärgiks oli uurida aju plastilisust ja ümberorganiseerumist erineva vaskulaarse geneesiga perinataalset insulti põdenud lastel, kasutades funktsionaalset MRT-d (fMRT), leidmaks seoseid laste motoorse, keele-

lise ja kognitiivse kaugtulemi ning aju struktuursete ja funktsionaalsete võrgustike muutuste vahel. Täpsemad eesmärgid olid:

- Hinnata aju subkortikaalsete struktuuride – basaaltuumade, talamuse ja *hippocampus*'e – mahtusid perinataalse insuldi põdemise järgselt ning leida seoseid subkortikaalsete struktuuride mahtude ja käe motoorika vahel (Uuring I).
- Leida erinevusi AIId ja PVId põdenud laste aju rahuoleku funktsionaalsetes võrgustikes ja kognitiivses arengus ning võrrelda saadud tulemusi tulemustega tervetel lastel (Uuring II).
- Leida erinevusi perinataalset AIId põdenud ja hilise diagnoosiga PVIga laste keelelise võimekuse ja aju stimuleerimisel kõnekeskuste lateraliseerumisel ning võrrelda saadud tulemusi tulemustega tervetel lastel, kasutades funktsionaalset magnetresonantstomograafilist uuringut (Uuring III).

11.3 Uuritavad ja meetodid

Uuringutesse kaasati PVId ja AIId põdenud 7-18-aastased lapsed Eesti laste insuldi andmekogust (ELIA), kes olid sündinud ajalistena (≥36 rasedusnädalat) ja ilma teiste kesknärvisüsteemi haigusteta. Perinataalset insulti põdenud lapsi võrreldi soo ja vanuse poolest sobivate tervete lastega. Kõigilt uuringus osalenud lastelt ja nende vanematelt saadi kirjalik informeeritud nõusolek.

Insulti põdenud laste üldist arengut ning neuroloogilist leidu hindasid lasteneuroloogid, kasutades selleks spetsiaalselt insulti põdenud laste jaoks väljatöötatud skaalat: *Pediatric Stroke Outcome Measurement* (PSOM) (Kitchen et al., 2012). Laste käelist motoorset funktsiooni hinnati *Assisting Hand Assessment* (AHA) (Krumlinde-Sundholm et al., 2007) ja *Manual Ability Classification System* (MACS) (Eliasson et al., 2006) testidega.

Laste kognitiivset arengut hindas kliiniline psühholoog, kasutades *Kaufman Assessment Battery for Children II* (K-ABC-II) testipatareid (Kaufman and Kaufman, 2004). Testi alusel hinnati laste üldist kognitiivset võimekust (Fluid-Crystallized Index (FCI), Mental Processing Index (MPI) ja Nonverbal Index (NVI)) ja võimeid erinevates skaalades: samaaegne infotöötlus, järjestikune infotöötlus, planeerimine, õppimine ja teadmised. Laste kõnelis-keelelistest võimetest ülevaate saamiseks kasutati kolme alltesti: sõnavara (EV), verbaalsed teadmised (VK) ja mõistatuse (RID) näitajad.

3D T1 kaalutud kujutised ning rahuoleku ja stimuleeritud fMRT uuringud teostati 3T Philips Achieva MRT skanneriga. Rahuoleku fMRT pildiseeria abil uuriti aju rahuoleku võrgustikke 6 minuti jooksul. Kahes stimuleeritud fMRT pildiseerias kasutati suulisi keelelisi ülesandeid: tegusõnade tuletamist või lausete tõesuse hindamist vaheldumisi kontrollülesannetega, ühe käe sõrmede nipsutamisega, kokku 6 minuti ja 40 sekundi jooksul.

MRT uuringud anonümiseeriti ja arvutused tehti TÜ Teadusarvutuste keskuses FMRIB software library's (FSL) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) tarkvaraga. Rahuoleku fMRT võrgustikud leiti, kasutades FSLi MELODIC tööriista ja *Independent component analysis* (ICA) töövoogu (Uuring I). Subkorti-

kaalsete struktuuride mahud leiti poolautomaatselt, rakendades automaatset FSLi FIRST tööriista ja manuaalset korrigeerimist, kasutades FSLeyes programmi (Uuring II). Mahud normeeriti FSLi SIENAX programmi abil arvutatud lapse pea suurusega. Laste kõnekeskuste aktivatsioon arvutati FSLi FEAT tööriistaga, lateralisatsioon Broca ja Wernicke piirkondades arvutati Matlabi SPM keskkonnas LI-toolbox tööriistaga (Uuring III) (Wilke and Lidzba, 2007; Wilke and Schmithorst, 2006).

AIId ja PVId põdenud laste motoorikat ja kognitiivseid võimeid, aju rahuoleku fMRT võrgustikke, subkortikaalsete struktuuride mahtu ja kõnekeskuste lateralisatsiooni võrreldi vastavate näitajatega tervetel lastel, kasutades statistika programme SAS ja R. Perinataalset insulti põdenud laste subkortikaalsete struktuuride mahtusid korreleeriti motoorikaga ning nende keelelisi võimeid korreleeriti kõnekeskuste lateralisatsiooniga.

11.4 Tulemused

Uurisime aju subkortikaalsete struktuuride mahtude seost motoorikaga, lõplikus uuringurühmas oli 7 neonataalse AII, 9 hilise diagnoosiga AII ning 18 hilise diagnoosiga PVIga last ja 42 tervet kontrollgrupi last vanuses 4-18 aastat. Nii AIId kui PVId põdenud laste ajukahjustuse vastaspoolse käe motoorika oli piiratud nii AHA kui MACS testi järgi, kuid grupid üksteisest statistiliselt oluliselt ei erinenud.

AIId põdenud laste mõlemad talamused, kahjustusepoolne kahkjaskeha (*globus pallidus)*, hipokampus *(hippocampus*) ja naalduv tuum (*nucleus accumbens)* olid tervete laste vastavate struktuuridega võrreldes väiksemad. AIId põdenud lastel korreleerusid suurem kahjustusepoolne talamus, koorik (*putamen*), kahkjaskeha, hipokampus, mandelkeha (*amygdala*) ja kahjustuse vastaspoolne mandelkeha parema tulemusega AHA testi alusel. AIId põdenud laste ajus kahjustuse vastaspoolse kooriku ja hipokampuse suurenemine oli seotud halvema tulemusega AHA testi alusel.

PVId põdenud lastel olid kahjustusepoolne talamus, sabatuum, kahkjaskeha ja hipokampus tervete laste vastavate struktuuridega võrreldes väiksemad. PVId põdenud lastel ei olnud kahjustuse vastaspoolel muutusi võrreldes tervete lastega. PVId põdenud laste suurem kahjustusepoolne talamus, sabatuum ja kahjustuse vastaspoolne talamus olid korrelatsioonis parema tulemusega AHA testil.

Rahuoleku fMRTle keskendunud uuringus oli uuringurühmas 7 vasakpoolset AIId põdenud ja 10 vasakpoolset PVId põdenud last ning 19 tervet last vanuses 7,6 kuni 17,9 aastat. Võrreldes tervete lastega oli AIId põdenud laste kognitiivne võimekus halvem ($p < 0{,}01$) kõigi K-ABC-II näitajate osas välja arvatud õppimisvõime, ja halvem ka võrreldes PVId põdenud laste kõigi kolme K-ABC-II üldvõimekuse näitaja, FCI, MPI ja NVI, osas ($p < 0{,}05$). PVId põdenud laste kognitiivne võimekus oli K-ABC-II järgi tervete lastega võrreldes kehvem üldise kognitiivse võimekuse (FCI) ning samaaegse ja järjestikuse informatsiooni töötlemise ($p < 0{,}05$) näitajate osas.

Näitasime 2016. a esimesena maailmas, et perinataalset insulti põdenud lastel on võimalik teostada rahuoleku fMRTd. ICAga arvutatud 30st võrgustikust 13 olid erinevates gruppides stabiilsed ja seostatud kindla funktsionaalsusega. Leidsime, et *default mode network*, mis aktiveerub enesepeegelduse ja sisekaemuse ajal, jaguneb antud vanuserühmas veel kaheks – eesmiseks ja tagumiseks võrgustikuks. Tagumine ja olulisem *default mode network* võrgustiku osa oli AIId põdenud lastel mahuliselt suurem võrreldes tervete laste omaga ($p < 0,01$).

Kolmandas uuringus võrdlesime perinataalset insulti põdenud laste kõnekeskuse lateralisatsiooni ja keelelisi oskusi. Lõplikus uuringurühmas oli vasakpoolse kahjustusega 9 AIId ning 12 PVId põdenud ja 30 tervet kontrollgrupi last vanuses 7,3 kuni 18,7 aastat. Perinataalset insulti põdenud laste kognitiivsed ja keelelised võimed olid võrreldes tervete lastega halvemad. AIId põdenud laste EV oli võrreldes PVId põdenud lastega kehvem ($p = 0,0284$). Väikese AII kahjustuskoldega laste EV oli võrreldes suure kahjustuskoldega AIId põdenud laste EVst parem ($p < 0,05$, Mann–Wilcoxon–Whitney test).

AIId ja PVId põdenud laste kõnevõrgustike lateralisatsioon erines tervete laste omast. Enamiku PVId põdenud laste kõnekeskus oli sarnaselt tervete lastega lateraliseerunud vasakule poole. Suure AII kahjustuskoldega laste kõnekeskus oli ümberorganiseerunud ja lateraliseerunud paremale poole, samas kui väikese, ainult kortikaalse kahjustuse korral oli kõnekeskus tavapärasel vasakul poolel ($p = 0.037$). Erinevate keeleliste fMRT ülesannete korral võis samal lapsel lateralisatsioon erineda.

Kõnekeskuste vasakpoolne lateralisatsioon tegusõnade tuletamise fMRT testi ajal oli korrelatsioonis paremate kognitiivsete võimetega nii PVId kui AIId põdenud lastel, aga mitte tervetel lastel. Sõnade tuletamise fMRT testi aegne vasakpoolsem lateralisatsioon korreleerus AIId põdenud lastel parema EV alatesti ja PVId põdenud lastel parema VK, EV ja RID keeleliste alatestide tulemustega K-ABC-II testipatarei järgi. Uuringust selgus, et kõnekeskuse reorganiseerumine paremasse hemisfääri suure kahjustuskoldega AII korral kompenseerib kahjustust, aga ei taga täielikult normaalset kõnefunktsiooni.

Kõik uuringud olid mõjutatud sarnastest uuringurühma piirangutest. Perinataalset insulti põdenud laste ajupildi analüüsi tegid keeruliseks suured kahjustusejärgsed muutused ja perinataalset insulti põdenud laste uuringugrupi suur vanusevahemik. Kuna perinataalset insulti põdenud lastel puudusid taastusravialased andmed, ei olnud võimalik nende mõju aju arengule ja motoorsele arengule hinnata.

11.5 Järeldused

1. Perinataalset insulti põdenud laste aju subkortikaalsete struktuuride mahud erinevad tervete laste omadest. Nii AIId kui ka PVId põdenud laste kahjustusepoolse talamuse mahu vähenemine toob kaasa käe funktsiooni häirumise, kusjuures basaaltuumade mahu seosed käe motoorikaga olenevad insuldi tüübist.

2. Perinataalset insulti põdenud laste aju rahuoleku funktsionaalsed võrgustikud ja kognitiivne võimekus erinevad tervete laste omadest. Perinataalset insulti põdenud laste kognitiivsed võimed olenevad kahjustuse vaskulaarsest tüübist ja AIId põdenud laste kognitiivsed võimed on halvemad võrreldes PVId põdenud laste vastavate näitajatega.
3. Perinataalset insulti põdenud laste keeleline võimekus oleneb insuldi tüübist ja on kehvem võrreldes tervete laste keelelise võimekusega.
4. Kõnekeskuste lateralisatsioon oleneb insuldi tüübist ja kahjustuse suurusest ning kõnekeskuste ümberorganiseerumine paremasse ajupoolkerasse on seotud kehvema keelelise võimekusega.

Insuldi kahjustuse vaskulaarse tüübi, kahjustuse suuruse ja subkortikaalsete struktuuride haaratuse radioloogiline hindamine MRTs on oluline perinataalset insulti põdenud laste motoorse, keelelise ja kognitiivse võimekuse prognoosi hindamisel. Täpne vaskulaarse kahjustuse ja struktuuride haaratuse hindamine aitab neuroloogidel ja teistel spetsialistidel hinnata taastusravi vajadust ja ulatust – õigeaegne ning personaliseeritud taastusravi tagab parema elukvaliteedi nii patsiendile kui ka tema lähedastele.

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13 PUBLICATIONS

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Publications:

1. Ilves, Nigul; Lõo, Silva; Ilves, Norman; Laugesaar, Rael; Loorits, Dagmar; Kool, Pille; Talvik, Tiina; Ilves, Pilvi (2022). Ipsilesional volume loss of basal ganglia and thalamus is associated with poor hand function after ischemic perinatal stroke. BMC Neurol. 22, 23. https://doi.org/10.1186/s12883-022-02550-3
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1. Ilves, Nigul; Lõo, Silva; Ilves, Norman; Laugesaar, Rael; Loorits, Dagmar; Kool, Pille; Talvik, Tiina; Ilves, Pilvi (2022). Ipsilesional volume loss of basal ganglia and thalamus is associated with poor hand function after ischemic perinatal stroke. BMC Neurol. 22, 23. https://doi.org/10.1186/s12883-022-02550-3
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