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Department of Radiology, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia.

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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, Norman; Laugesaar, Rael; Rull, Kristiina; Metsvaht, Tuuli; Lintrop, Mare; Laan, Maris; Loorits, Dagmar; Kool, Pille; Ilves, Pilvi (2022). Maternal Pyelonephritis as a Potential Cause of Perinatal Periventricular Venous Infarction in Term-Born Children. J Child Neurol. 2022 Aug; 37(8–9): 677–688. doi: 10.1177/08830738221109340. Epub 2022 Jul 12.

II. Ilves, Norman; Metsvaht, Tuuli; Laugesaar, Rael; Rull, Kristiina; Lintrop, Mare; Laan, Maris; Loorits, Dagmar; Kool, Pille; Ilves, Pilvi (2024). Periventricular hemorrhagic infarction in preterm neonates: etiology and time of development. J Neonatal Perinatal Medicine. Pre-press, pp. 1–11. 2024. doi: 10.3233/NPM-23033. Epub 2024 Jan 4.

III. Ilves, Norman; Pajusalu, Sander; Kahre, Tiina; Laugesaar, Rael; Šamarina, Ustina; Kool, Pille; Ilves, Pilvi (2023). High prevalence of collagenopathies in preterm and term born children with periventricular venous hemorrhagic infarction. J Child Neurol. 2023 May; 38(6–7): 373–388. doi: 10.1177/08830738231186233. Epub 2023 Jul 10.

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

2. ABBREVIATIONS

AIS	arterial ischemic stroke
aOR	adjusted odds ratio
CI	confidence interval
CT	computed tomography
EPSD	Estonian Pediatric Stroke Database
FDR	false discovery rate
GTI	genitourinary tract infection
GMH	germinal matrix hemorrhage
GW	gestational week
HS	hemorrhagic stroke
HR	hazard ratio
IQR	interquartile range
IVH	intraventricular hemorrhage
MRI	magnetic resonance imaging
MTHFR	methylenetetrahydrofolate reductase mutations
NA	not available
OR	odds ratio
PSOM	pediatric stroke outcome measure
PVHI	periventricular hemorrhagic infarction
PVI	periventricular venous infarction
SD	standard deviation
SNAPPE	score for neonatal acute physiology and SNAP perinatal extension
SVT	sinovenous thrombosis
US	ultrasonography

3. INTRODUCTION

Stroke is one of the leading causes of death and disability in adults (Donkor, 2018), but stroke occurs and leads to disability also in childhood, including the fetal and neonatal periods (Kirton and deVeber, 2013; Fluss et al., 2019; Dunbar et al., 2020). Perinatal stroke encompasses a heterogeneous group of cerebrovascular focal neurological injuries early in brain development, which probably affects more than 5 million people worldwide (Dunbar et al., 2020).

In recent years the incidence of perinatal stroke has increased due to improved awareness among child neurologists, higher availability of magnetic resonance imaging (MRI) and better recognition of perinatal stroke among radiologists (Chalmers, 2005; Dunbar et al., 2020). However, perinatal stroke is probably still an underdiagnosed condition, especially in mild cases (Dunbar et al., 2020). The total estimated birth prevalence of perinatal stroke in term born children is 1:1100 (Dunbar et al., 2020).

Perinatal stroke involves patients with different subtypes with a different etiology, pathogenesis and location (Dunbar and Kirton, 2018), and evolves at different stages of vascular development of the brain during fetal and neonatal life (Fluss et al., 2019). Radiological imaging allows to determine the vascular type and size of perinatal stroke even in the chronic stage if diagnosed after the neonatal period.

Arterial ischemic stroke (AIS) is the most frequent type of perinatal stroke in term born children diagnosed in the neonatal period; hemorrhagic stroke (HS) and sinovenous trombosis (SVT) are rare in the perinatal period (Kirton et al., 2008; Fluss et al., 2019; Clive et al., 2020; Dunbar et al., 2020). Some neonates have no neurological symptoms after birth, but spastic hemiparesis and/or focal seizures appear during infancy. In these infants brain MRI reveals most frequently periventricular venous infarction (PVI), more rarely AIS (Ilves et al., 2016; Dunbar et al., 2020).

In preterm born neonates the most frequent type of stroke is periventricular hemorrhagic infarction (PVHI) (Roy et al., 2022) with adverse long-term outcome. Worldwide 13.4 Million newborns (9.9% of all births) were born preterm at gestational week (GW) <37 without a measurable decrease in preterm birth rates from 2010 to 2020. Of all preterm births 15% occurred at less than GW 32 and these neonates are at high risk for periventricular hemorrhagic infarction (PVHI) (Ohuma et al., 2023). The economic burden of prematurity and its complications is high. In the United States, the annual economic burden of preterm birth was estimated at 26 billion US dollars in 2005 (Allen, 2008).

Maternal transport, antenatal steroid and postnatal surfactant administration, as well as improved resuscitation and intensive care techniques have become the standard of care for premature infants worldwide, increasing the survival of the smallest premature infants who show relatively high incidence of intraventricular hemorrhage (IVH) or PVHI with poor neurodevelopmental outcome (Allen, 2008; “Volpe’s Neurology of the Newborn – 6th Edition,” n.d.; Stoll et

al., 2010; Handley et al., 2018). However, the incidence of severe (grade III-IV) IVH and PVHI remained high and decreased only from 9.4 % to 7.9% in the Vermont Oxford Network (including 90% of all very low birth-weight infants (501-1500g) in the United States) among children below GW 30 or birth weight 401-1500g between 2005 and 2014(Stoll et al., 2010; Horbar et al., 2017).

Children with perinatal stroke carry a high risk for permanent lifelong impairment and they have often combined disabilities including motor, cognitive and language impairment, epilepsy, behavioral problems, attention deficit, hyperactivity disorder and academic difficulties (Mercuri, 2003; Jenks et al., 2009, 2009; Kirton and deVeber, 2013; Whittingham et al., 2014; Kuczynski et al., 2016; Lõo et al., 2018; Dunbar and Kirton, 2018; Ferriero Donna M. et al., 2019; Hawe et al., 2020; Virani et al., 2022). Perinatal stroke usually results in reduced ability to achieve functional independence rather than its loss, and some problems like academic difficulties and cognitive deficit may evolve as the child matures and higher academic performance is expected (Westmacott et al., 2009). The lifelong individual, family, and societal burdens of perinatal stroke are high as children with stroke have to live their whole life with a disability. Permanent lifelong disability may lead to depression and stress for patients and their families (Bemister et al., 2015).

It is essential to promote early diagnosis and treatment of perinatal stroke and early rehabilitation of children with expected lifelong disability. Yet rehabilitation can merely alleviate disabilities to improve the quality of life of the patients, but it cannot reverse the whole damage in the developing brain. As rehabilitation can only reduce the damage, the main objective should be avoiding stroke.

Only a few studies have investigated the etiology of presumed antenatal PVI in term born children, diagnosed after the neonatal period, with probably antenatal development of stroke. There is no clear understanding of why and when exactly presumed antenatal PVI occurs during pregnancy and it has only been suggested that it occurs before 34 GW (Kirton et al., 2010; Ilves et al., 2016; Dunbar and Kirton, 2018). Furthermore, information about risk factors for antenatally diagnosed stroke is insufficient (Özduman et al., 2004; Elchalal et al., 2005; Dunbar et al., 2021). Without the knowledge of a clear etiology, it is difficult to avoid antenatal development of stroke.

Literature data support the multifactorial etiology of severe IVH and PVHI in preterm born children (Ment et al., 2015). The bulk of research on their etiology and pathogenesis is based on risk factors during delivery and intensive care ("Volpe's Neurology of the Newborn – 6th Edition," n.d.). However, in some preterm born children severe IVH/PVHI is detected already within the first hours of birth, or PVHI is even in the state of involution at birth and is considered to be antenatal (de Vries et al., 1998; Harteman et al., 2012). A better understanding of the maternal risk factors that can lead to antenatal development of PVHI can facilitate prevention of severe IVH/PVHI in preterm born children (Dunbar et al., 2021).

Emerging data suggest that various gene variants may contribute to development of severe hemorrhage and antenatal stroke in preterm born children. It has even beem suggested that genetic predisposition might be the leading factor for development of PVHI in preterm norn children(Ment et al., 2014, 2015). In a previous study of children with neonatal AIS, presumed AIS, and presumed antenatal PVI the thrombophilia risk was not higher (Curtis et al., 2017). High prevalence of collagenopathies has been found in fetuses with multifocal hemorrhagic and/or ischemic cerebral lesions (Matsumoto et al., 2015; Maurice et al., 2021). Genetic profiling has not been applied for evaluating collagenopathies among preterm and term born children with PVHI/PVI, although collagenopathies can lead to microangiopathy and cause recurrent hemorrhages (Knaap et al., 2006).

I started the study as a neurological and radiological outcome study of children with PVHI/PVI, but I soon understood that there is a gap in our knowledge of what is more important for the parents of children with stroke: why did my child develop stroke? Therefore, I refocused my study on the etiology of PVHI/PVI and work on outcome in these children will continue.

4. LITERATURE REVIEW

4.1. Definitions and classifications

By definition **perinatal stroke** is a heterogeneous condition with a brain injury due to focal cerebral arterial or venous occlusion that occurs between 20 weeks of fetal life through 28th postnatal day, and the diagnosis is confirmed by neuroimaging or neuropathology studies (Lynch et al., 2002; Raju et al., 2007; Lynch, 2009; Fluss et al., 2019). The diagnosis of perinatal stroke is often based on the neuroradiological confirmation of a vascular event; the clinical presentation of a stroke can be variable or mild symptoms can be overlooked.

Perinatal stroke is also classified, based on the time of detection of stroke, into antenatal, neonatal and presumed perinatal stroke (Figure 1). Antenatal stroke is diagnosed before birth, neonatal acute stroke is diagnosed during the first 28 days after birth and presumed perinatal stroke is diagnosed after the neonatal period if symptoms develop after that period.

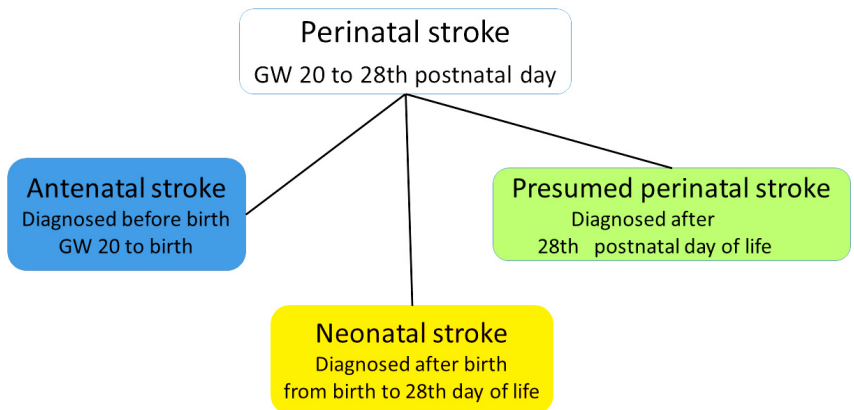

Figure 1. Classification of perinatal stroke based on the time of detection of stroke

According to the vascular type, perinatal stroke is divided into AIS, hemorrhagic stroke (HS), sinovenous thrombosis (SVT) and PVI based on acute or chronic findings on MRI (Fluss et al., 2019; Dunbar et al., 2020). According to Dunbar and coworkers, six perinatal stroke syndromes can be defined (Dunbar et al., 2020). Neonatal acute AIS is defined as presence of one or more areas of restricted diffusion within an arterial territory on MRI. Neonatal acute SVT is defined as presence of thrombus within a cerebral vein or sinus confirmed by magnetic resonance venography or CT venography or, if thrombus cannot be definitively demonstrated, a pattern of venous infarction within a known cerebral venous territory. Neonatal acute HS is defined as a focal collection of acute or subacute blood within the brain parenchyma with or without intraventricular

or subarachnoid blood. If stroke is detected in the chronic stage after the neonatal period, chronic-stage presumed AIS is defined as a chronic focal encephalomalacia attributable to remote infarction in an arterial territory; presumed HS shows evidence of remote intraparenchymal hemorrhage on imaging. Term born children with presumed antenatal PVI have chronic focal encephalomalacia of the periventricular white matter with sparing of the deep gray matter and cortex with additional supportive evidence of remote germinal matrix hemorrhage (GMH) demonstrated on susceptibility weighted imaging.

In preterm born children acute periventricular hemorrhagic infarction (PVHI) occurs in association with large IVH causing perientricular venous infarction and leading to porencephalic periventricular cysts communicating often with the lateral ventricle in the chronic stage ("Volpe's Neurology of the Newborn – 6th Edition," n.d.).

4.1.1. Classification according to the vascular type

Most commonly, **term born children** with perinatal stroke have AIS or PVI (Kirton et al., 2008; Ilves et al., 2016; Kitai et al., 2016; Dunbar and Kirton, 2019; Dunbar et al., 2020). Among 219 cases of perinatal stroke in a population-based study, neonatal or presumed AIS was diagnosed in 49 % of the cases, PVI in 21% of the cases, neonatal or presumed HS in 18% of the cases and sinovenous thrombosis in 12% of the cases (Dunbar et al., 2020).

Acute AIS typically presents within the first 28 days of life and is confirmed by neuroimaging with the focal area of a recent ischemic infarction, corresponding to one or more arterial territories, in 95% of cases in the medial cerebral artery (Kirton et al., 2008; Dunbar and Kirton, 2019; Dunbar et al., 2020). The most common presentation is seizures (either focal or generalized) occurring 12–72 hours following delivery, observed in 70–90% of infants (Dunbar and Kirton, 2019; Fluss et al., 2019).

Based on the classification of Kirton and co-authors (Kirton et al., 2008)**,** PVI in term born children is described as a medullary venous territory infarction in the periventricular area, with a porencephalic enlargement of the lateral ventricle, and/or periventricular gliosis and/or cystic changes, while the cerebral cortex is spared; hemosiderin may be present in the area of the germinal matrix, in the ventricles and/or in periventricular lesion (depending on the time of imaging) (Takanashi et al., 2003; Kirton et al., 2008; Ilves et al., 2016; Fluss et al., 2019). It is suggested that presumed antenatal PVI develops in utero (before GW 34) after GMH or IVH leading to the compression of the medullary veins and development of focal venous infarction of the periventricular white matter (Fluss et al., 2019).

HS and SVT are more rare in term born children (Kirton et al., 2008; Clive et al., 2020; Dunbar et al., 2020) and are not the focus of this study.

Common vascular types of stroke described in **preterm born children** are PVHI accounting for 80% of cases, perinatal AIS accounting for 17% and SVT accounting for 3% (Roy et al., 2022).

PVHI is caused by GMH or IVH, which leads to periventricular venous thrombosis and infarction, mostly known in preterm children born around gestational weeks 24–34 (de Vries et al., 2001; Fluss et al., 2019). GMH/IVH is described with grades 1–4: grade I – hemorrhage in the germinal matrix; grade II – intraventricular blood without distension of the ventricular system; grade III – blood filling and distending the ventricular system without visible periventricular stroke on ultrasound; and grade IV – previously thought as parenchymal involvement of IVH (Papile et al., 1978; Szymonowicz and Yu, 1984; "Volpe's Neurology of the Newborn – 6th Edition," n.d.). In 8–15 % of cases, GMH/IVH is complicated with PVHI, previously referred to as grade IV IVH ("Volpe's Neurology of the Newborn – 6th Edition," n.d.; Inder et al., 2017; Handley et al., 2018; eurUS.brain group et al., 2020). For preterm neonates, the term periventricular hemorrhagic infarction is used to emphasize the hemorrhagic component of PVHI ("Volpe's Neurology of the Newborn – 6th Edition," n.d.).

PVHI is common among extremely premature infants and in children with very low birth weight. In children with birth weight less than 1000g or GW 28, PVHI can occur in almost 15 to 20 % of all children with IVH (Perlman and Volpe, 1986; Golden et al., 1997; Darlow et al., 2003; Wilson-Costello et al., 2005; "Volpe's Neurology of the Newborn – 6th Edition," n.d.). The last population based study in California showed a decrease in the incidence of grade III IVH/PVHI (severe IVH/PVHI) children born 22–31^{+6} GW, from 9.7% in 2005 to 5.9% in 2015 (Handley et al., 2018). In Estonia the population based audit of preterm deliveries at GW 22–31^{+6} in 2015–2016 revealed severe IVH/PVHI in 7% of children (https://www.tervisekassa.ee/partnerile/tervishoiuteenuste-kvaliteet/kliinilised-auditid#tab-auditid-2019), which demonstrates a gradual decrease compared to 2007–2008 and 2002–2003, when the incidence of severe IVH/PVHI in a similar population of Estonia was 12% and 15%, respectively (Zeitlin et al., 2016). In extremely premature children with birth weight under 750g the risk for any grade IVH is three times higher than for preterm infants with birth weight over 1250g (42% vs.14%) (Perlman and Volpe, 1986; Wilson-Costello et al., 2005; "Volpe's Neurology of the Newborn – 6th Edition," n.d.).

Neuropathological studies show that PVHI is not a mere sign of intraventricular blood extending into the periventricular white matter, as was thought earlier, but indicates periventricular venous infarction with hemorrhagic necrosis (Takashima et al., 1986; Gould et al., 1987). The underlying mechanism of PVHI is periventricular venous congestion and obstruction induced by GMH/IVH, leading to ischemia and secondary periventricular hemorrhagic infarction (Perlman and Volpe, 1986; Takashima et al., 1986; de Vries et al., 2001; "Volpe's Neurology of the Newborn – 6th Edition," n.d.; eurUS.brain group et al., 2020). The higher is the grade of GMH/IVH, the more likely it is that PVHI will develop (Bassan et al., 2006a; Inder et al., 2017; eurUS.brain group et al., 2020), hence PVHI develops on the side of a larger GMH or IVH ("Volpe's Neurology of the Newborn – 6th Edition," n.d.). High intraventricular pressure due to large hemorrhage in higher grade IVH additionally affects the blood flow through the subependymal veins, increasing the size of the infarct (eurUS.brain

group et al., 2020). PVHI is mostly unilateral (90%), associated most often with ipsilateral grade III IVH (84%), and located in the parietal lobe (51%) (Cizmeci et al., 2020).

The obstruction of the venous drainage of the periventricular tissues by germinal layer haemorrhages leads to PVHI and periventricular necrosis (Gould et al., 1987). A single large periventricular cyst develops in 66% of cases and a large cyst is combined with smaller cysts in 23% of cases (Bassan et al., 2006a). Cystic lesion communicates often but not always with the lateral ventricle (Bassan et al., 2006a) causing ventriculomegaly. Associated posthemorrhagic ventricular dilation occurs in 36% of PVHI patients with poor cognitive and motor outcome (Cizmeci et al., 2020).

4.1.2. Classification according to the time of diagnosis

Antenatal stroke is defined as an ischemic, thrombotic, or hemorrhagic event occurring between GW 14 and the onset of labor resulting in delivery (Özduman et al., 2004; Govaert, 2009). Antenatal IVH or some other type of intracerebral hemorrhage or PVHI is usually detected by antenatal ultrasonography (US), if known maternal or fetal risk factors are found and the diagnosis is confirmed with antenatal or postnatal MRI or autopsy. Prenatal imaging, most optimally MRI, has shown hemorrhagic lesions in over 90% of investigations; porencephalies have been identified in 13% (Özduman et al., 2004). Antenatal stroke has various patterns of injury on neuroimaging, including hemorrhage or ischemic lesion leading to multicystic encephalomalacia, porencephaly, schizencephaly or cortical malformation depending on gestational age at the time of stroke (Hino-Fukuyo et al., 2016).

A recent metaanalysis of children with antenatal IVH or PVHI showed that grade I/II GMH-IVH was present in 11.6%, grade III in 43.1%, and PVHI in 45.3% of the total of 240 children, 53% of them were born at term and 47% were born preterm (Dunbar et al., 2021).

Antenatal stroke is an important cause of cerebral palsy and 78–92% of cases with reported outcome resulted in either death or adverse neurodevelopmental outcome (de Vries et al., 1998; Özduman et al., 2004; Dunbar et al., 2021). In an earlier case series of unilateral spastic cerebral palsy in term born infants, insult occurred antenatally in at least one third of cases including unilateral periventricular leucomalacia and structural malformation such as porencephaly and schizencephaly (Steinlin et al., 1993). Vascular disruption secondary to focal ischemia or hemorrhage appears to play a role in development on schizencephaly and porencephaly (Steinlin et al., 1993).

Neonatal stroke is diagnosed from birth up to 28 days of life; the child shows acute symptoms during the neonatal period, since this type of stroke develops around birth (Raju et al., 2007; Fluss et al., 2019; Dunbar and Kirton, 2019; Dunbar et al., 2020). Symptoms occurring during the neonatal period are mostly well recognized after term birth with typically developing seizures within 12–72 hours of life and typical findings of acute stroke on MRI

(Laugesaar et al., 2007; Raju et al., 2007; Dunbar and Kirton, 2018, 2019; Fluss et al., 2019). Term born children with perinatal stroke develop acute neonatal AIS in 37%, neonatal HS in 16% and SVT in 12% of cases (Dunbar et al., 2020). Neonatal acute stroke (AIS; HS; SVT) in term born children is not discussed further in the present study (Figure 2).

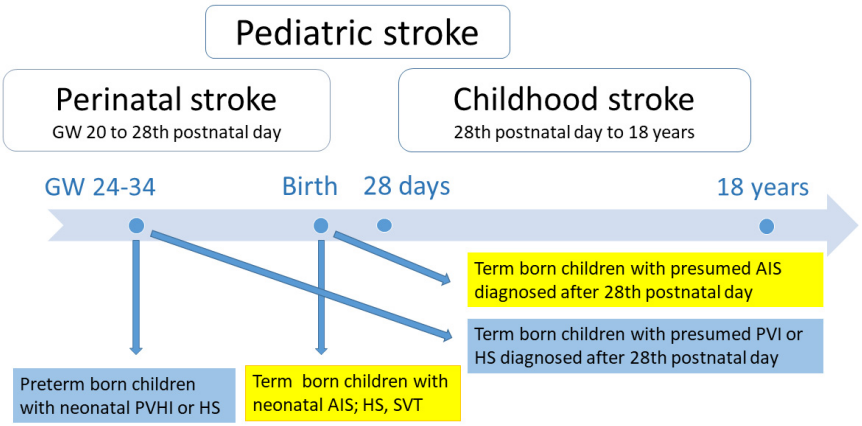

Figure 2. Time of the development and time of the diagnosis of PVHI/PVI is children with perinatal stroke. AIS, arterial ischemic stroke; HS, hemorrhagic stroke; PVHI, periventricular hemorrhagic infarction; PVI, periventricular venous infarction; SVT, sinovenous thrombosis

In preterm born children with neonatal stroke (Figure 2) lower grade GMH/IVH precedes PVHI and progression to higher grades IVH/PVHI occurs rapidly (de Vries et al., 2001; Bassan et al., 2006a; "Volpe's Neurology of the Newborn – 6th Edition," n.d.; eurUS.brain group et al., 2020). Although 34% of children show a normal initial US scan, half of affected children have GMH on the first day of life and 81–90% of PVHIs are diagnosed by the 3rd or 4th day of life, with a median age of 46 hours at diagnosis of PVHI (de Vries et al., 2001; Bassan et al., 2006a; eurUS.brain group et al., 2020). After the first week of life, progression of hemorrhage is rare irrespective of gestational age (de Vries et al., 2001; Bassan et al., 2006a; eurUS.brain group et al., 2020). In a study evaluating the time of occurrence of PVHI in preterm born children, 73% of typical PVHIs occurred between 6—96 h of age and atypical PVHI (<6hours or >96 h of age) occurred due to the factor V Leiden mutation and showed higher prevalence of cerebral palsy (Harteman et al., 2012).

Presumed perinatal stroke in term born children is diagnosed retrospectively, with neurologic deficit being discovered after the neonatal period (after postnatal day 28) and the focal chronic stage of infarction being recognized on neuroimaging (Raju et al., 2007) (Figure 2). These children with presumably antenatal stroke do not have neurological symptoms after birth, but spastic

hemiparesis and/or focal seizures appear during infancy (Ilves et al., 2016). In a population based study delayed presentation of perinatal stroke was noted in 36% (78 of 219) of children (Dunbar et al., 2020). Presumed PVI and AIS are diagnosed most frequently and presumed HS is rare (Kirton et al., 2008; Kitai et al., 2016; Ilves et al., 2016; Dunbar and Kirton, 2019; Fluss et al., 2019; Dunbar et al., 2020). Two-thirds of the cases of presumed perinatal stroke are due to PVI and one third of the cases are due to AIS (Kitai et al., 2016; Ilves et al., 2016).

4.1.3. Gestational age at the onset of stroke in preterm and term born children with PVHI/PVI

The incidence of PVHI increases with lower gestational age (Golden et al., 1997; Handley et al., 2018). Among children with a gestational age of 22–23 weeks severe IVH/PVHI) occurred in 36 %, but among children with a gestational age of 30–31 weeks, in only 1.2 % (Handley et al., 2018). The probable reason for the lower rate of GMH among children with higher gestational age is a progressive decrease in the size of the germinal matrix to nearly complete involution by GW 36, decreasing thus the likelihood of hemorrhage (Szymonowicz et al., 1984; Szymonowicz and Yu, 1984).

PVHI may develop already prenatally (de Vries et al., 1998, 2001; Özduman et al., 2004; Elchalal et al., 2005). Antenatal GMH/IVH of various severity is the most common type of fetal intracranial hemorrhage (Özduman et al., 2004; Elchalal et al., 2005), with median age at the diagnosis of antenatal IVH/PVH being GW 31 and median age at birth 37 weeks (range 25–41 GW) (Dunbar et al., 2021). In a prospective study of 1332 preterm children born at GW ≤34, the antenatal onset of hemorrhagic and/or ischemic lesions was found in 24 (1.8%) children, six of them had cystic lesions in the periventricular area; three more children had an established porencephalic cyst, indicating that antenatal PVHI may have reached the resolving phase by birth in these preterm born children (de Vries et al., 1998).

Although a similar underlying mechanism of both presumed antenatal PVI in term born children and PVHI in preterm born children has been agreed upon (Takanashi et al., 2003), the exact time of development of presumed antenatal PVI in utero in term born children is still unknown. Previously, it was only merely suggested that it takes place, like in preterm born children, from GW 24 to 34 (Fluss et al., 2019) or prior to 32 weeks (Dunbar and Kirton, 2019). This suggestion was based on the known pathophysiology of the prominent germinal matrix of the fetal brain at GW 32–34 (Kirton et al., 2010; Dunbar and Kirton, 2019; Fluss et al., 2019). Vascular insult at GW <24 without fully completed neuronal migration can lead to schizencephaly and after GW 24 to porencephaly (Curry et al., 2005; Govaert, 2009; Yoneda et al., 2012; Hino-Fukuyo et al., 2016; Ilves et al., 2020).

Presumed antenatal PVI in term born children without noticeable symptoms at birth and development of hemiplegia later in infancy is believed to represent

in utero occurrence of an acute version of PVHI in preterm born children (Dunbar and Kirton, 2019). Some fetuses with antenatal PVHI stabilize in utero and survive; PVHI remains undetected during delivery and they have no acute symptoms after an uneventful term birth (Ilves et al., 2016). Term born children with presumed antenatal PVI present symptoms after the neonatal period when hemiparesis or delayed developmental milestones appear.

Mild cases of hemiparesis may be missed, which leads to underdiagnosing of PVI (Özduman et al., 2004). The problem of underdiagnosing patients with PVI is universal, as mild cases of hemiparesis may be missed because of subtle or unspecific findings which are not investigated by MRI (Özduman et al., 2004; Fluss et al., 2019). However, owing to the better recognition of perinatal stroke among neonatologists and child neurologists, age at postnatal diagnosis and prevalence of presumed perinatal stroke have decreased over time (Dunbar et al., 2020).

Differences in prenatal risk factors between PVHI in preterm infants and presumed antenatal PVI in term born infants and, most importantly, the exact timing of the risk factor leading to infarction in utero in presumed antenatal PVI have not been clearly established (Fluss et al., 2019). Without the knowledge of risk factors and onset of their occurrence, prevention of stroke leading to lifelong impairments is impossible.

4.1.4. Neuroimaging of stroke

Neuroimaging, mainly brain US or MRI, is employed for the diagnosis of antenatal, neonatal, as well as presumed antenatal perinatal stroke. MRI usually confirms the diagnosis of perinatal stroke as it enables to distinguish stroke from other neurological diseases, like asphyxia and congenital infections, as well as from other congenital abnormalities. As the availability of MRI and the awareness of perinatal stroke among radiologists increase, some children with perinatal stroke with chronic changes are identified during MRI imaging done for mild neurological dysfunction. The location and size of stroke cause also differences in its clinical manifestation (Laugesaar et al., 2018; Lõo et al., 2018; Ilves et al., 2022b). The vascular type of stroke and the time of development of lesion can provide prognosis for outcome (Raju et al., 2007; Laugesaar et al., 2018; Ilves et al., 2022a, 2022b).

In the acute phase of PVHI in preterm born children, bedside cerebral US shows a parenchymal increase in echogenicity in the periventricular area, which can be fan-shaped and separate or partly communicating with the ventricle, and globular-shaped, communicating with the ventricle (Rademaker et al., 1994; de Vries et al., 2001; Bassan et al., 2006a; Dudink et al., 2008; Zagaglia et al., 2018; eurUS.brain group et al., 2020). In PVHI, usually unilateral cavitation develops within the periventricular white matter adjacent to the ventricular wall, and large porencephalic cyst or smaller periventricular cystic lesion is a common finding after 1 or 2 months (eurUS.brain group et al., 2020). However, loss

of tissue and cystic change can be visible after 2 weeks in severe cases (Fluss et al., 2019).

Confimative MRI in preterm born children is usually performed at a postmenstrual age of GW 40 or later; MRI diagnostics in the acute stage of PVHI in extremely preterm children is rare. Like preterm born children with PVHI at a postmenstrual age of GW 40, term born children with presumed antenatal PVI have chronic changes on MRI. Chronic changes of PVI/PVHI on MRI are similar, indicating a similar underlying mechanism of presumed antenatal PVI and PVHI in preterm children (Takanashi et al., 2003).

PVI in term born children is defined as unilateral medullary venous territory infarction in the periventricular area, with periventricular tissue loss and gliosis, focal or porencephalic enlargement of the lateral ventricle and T2-signal prolongation of the posterior limb of the internal capsule (Kirton et al., 2008, 2010). Compared to AIS, the cortex and the basal ganglia are usually relatively spared (Kirton et al., 2008; Fluss et al., 2019). Hemosiderin deposits might be visible in susceptibility weighted images in the region of the germinal matrix, in the ventricles and/or in periventricular lesion (Takanashi et al., 2003; Kirton et al., 2008; Kirton and Wei, 2010; Kirton et al., 2010; Fluss et al., 2019).

The severity of the findings of PVHI/PVI on MRI can be different, from very subtle with little deformation of the lateral ventricle and tissue loss of the corpus callosum, to much larger periventricular damage extending to the subcortical layer (Fluss et al., 2019). Children with presumed antenatal PVI have also a typical finding of the smaller size of the thalamus and basal ganglia, most often damage involving the nucleus caudatus (Ilves et al., 2022a).

4.2. Etiology of PVHI/PVI

4.2.1. Pregnancy risk factors in fetal death

Cerebrovascular accidents are common among stillborn infants. In an autopsy study of stillborn infants, 40% (70/175) of their brain showed evidence of ischemic cerebral injury, and in 62 of the 70 studied brains, the periventricular white matter was the main site of damage; in 39 cases ischemic cerebral lesions associated with the placenta showed evidence of infarction (Burke and Tannenberg, 1995). In another study of infants who were stillborn and/or died in the early neonatal period, a series of 165 brains was examined in order to identify the incidence and nature of prenatal brain damage. Of the stillborn infants, 44% showed evidence that brain damage was associated with circulatory disorders (Squier and Keeling, 1991). The most frequent abnormality of the stillborn infants was widespread ischemic damage to the white matter of the cerebral hemispheres in 26% of the cases; hemorrhage was seen in association with ischemic damage in 13% and brain hemorrhage without evidence of co–existing ischemic damage occurred in utero in 5% (Squier and Keeling, 1991). Of the 90 live–born infants, 12/75 (16%) of those who survived less than 3 days and three of

the 15 infants (20%) who lived 3 to 7 days after birth showed ischemic damage of prenatal origin (Squier and Keeling, 1991).

The incidence of fetal death in multiple gestation is 2.6%, the antepartum death of one fetus in multiple gestation can be associated with mortality or major morbidity for the survivor, (Carlson and Towers, 1989). Death of one twin increases the risk of vascular disruptions in the other and may lead to fetal or neonatal death, while twin-twin transfusion can be one of the underlying causes of the death of the donor (Carlson and Towers, 1989). Although the death of one fetus in the first trimester of twin pregnancy is not an uncommon event and does not have serious consequences for the survival of the other fetus, the death of one fetus in the second or third trimester of pregnancy is associated with dramatic increase in morbidity and mortality for the surviving co-twin. Death of one fetus after mid gestation has been associated with preterm labor, preeclampsia, intrauterine growth restriction, neurological complications or even the death of the surviving twin (Stefanescu et al., 2021).

4.2.2. Pregnancy and delivery risk factors associated with antenatal stroke

Although risk factors could not be assigned to 50% of the fetuses with antenatal stroke, the most common maternal conditions associated with antenatal stroke were alloimmune thrombocytopenia and trauma, according to a study by Özduman and coworkers (Özduman et al., 2004). Twin pregnancy is one of the most important risk factors for antenatal stroke (Kirkham et al., 2018). Cerebral palsy is more common in twins than in singletons, especially in co-twin death (Kirkham et al., 2018). Monochorionic diamniotic twins may be complicated by the twin-twin transfusion syndrome related to placental vascular anastomoses (Mackie et al., 2016) as one of the possible causes. Furthermore, genetic disorders like pathogenic *COL4A1* variants have been associated with fetal stroke (Vermeulen et al., 2011; Yoneda et al., 2013; Khalid et al., 2018; Hausman-Kedem et al., 2021a; Dunbar et al., 2021).

A recent metaanalysis of fantenatal IVH/PVHI children showed a variety of co-occurring conditions: 39% of children were from an uneventful pregnancy, 19% from a twin pregnancy, 11% were small for gestational age; other congenital anomalies occurred in 10%, poly- and oligoamnion in 7%, fetal bleeding diasthesis in 5%, maternal preeclampsia in 5%, non-infectious maternal illness in 4% and trauma in 3%; maternal infection, *COL4A1* mutation, diabetes, anticoagulation therapy and hypertension all occurred in 2% of cases (Dunbar et al., 2021). Fetal stroke was also associated with a history of fetal/neonatal alloimmune thrombocytopenia, pyruvate carboxylase deficiency, miscarriage, threatened preterm labor, placental abruption, parental thrombophilia, hydrops, neonatal liver disease, and fetal metabolic disease (Dunbar et al., 2021). Other studies have shown that fetal stroke can develop also in consequence of maternal cocaine use (Suchet, 1994), maternal warfarin and anticonvulsant use (Özduman et al., 2004), and carbon monoxide poisoning (Gul et al., 2009).

Bleeding diathesis has been the most frequently reported cause of fetal hemorrhage including GMH and IVH with venous infarction (Kirkham et al., 2018). The most common causes have been maternal (Özduman et al., 2004; Ito et al., 2009) or fetal (de Sousa et al., 1988) alloimmune thrombocytopenia, but also maternal idiopathic thrombocytopenia and fetal von Willebrand disease (Kirkham et al., 2018). Fetal prothrombotic disorders like homozygous protein C deficiency have also been reported as the cause of IVH with venous infarction (Kirkham et al., 2018).

4.2.3. Pregnancy and delivery risk factors for presumed antenatal PVI in term infants

There have been few controlled studies of the specific risk factors of stroke in the perinatal period in term born children, such as maternal characteristics, placental disorders, and other complications of pregnancy and birth. These are mostly reported for perinatal AIS (Lee et al., 2005; Lynch, 2009). The focus in term born neonates with perinatal stroke has also been on risks at delivery, while most of the research has been directed to AIS, which usually has neonatal symptoms. Case control studies focusing on neonatal AIS have identified four sets of risk factors: male sex, obstetric determinants (first pregnancy and caesarean section), birth hypoxia and fetal/neonatal inflammatory state (Giraud et al., 2017).

Term born children with presumed antenatal stroke are without specific symptoms at birth and only a few studies focus on the mechanisms of development of stroke during pregnancy. Previous research on the risk factors for presumed perinatal stroke has focused mainly on presumed AIS, while studies on presumed PVI have been relatively scarce and carried out on small cohorts. In some studies AIS and PVI children with presumed perinatal stroke have been combined and the authors suggest that prothrombotic and vascular causes should be ruled out (Gacio et al., 2015). It has also been recommended to rule out congenital heart diseases, as cardiac embolism is one of the known causes of perinatal stroke, followed by idiopathic causes (Lynch, 2009). However, the vascular genesis of presumed AIS and PVI is different (Kirton et al., 2010; Gacio et al., 2015).

Preeclampsia, maternal hypertension, recurrent miscarriage, antepartum bleeding and prenatal infection were earlier suggested to be associated with presumed PVI, however, none of these factors were evaluated in case-control studies (Kirton et al., 2010). Antepartum conditions such as preeclampsia, infections and past history of miscarriage are found more frequently in PVI than in neonatal AIS (Fluss et al., 2019; Ilves et al., 2016). A study of risk factors in 12 children with presumed PVI and in 47 cases with presumed AIS found a trend for increased chronic maternal factors (hypertension, recurrent miscarriage, antepartum bleeding and prenatal infection) for PVI (50%) compared with presumed AIS (32%), p=0.2 (Kirton et al., 2010). Antepartum conditions are in line with the presumed pathogenesis of PVI, which corresponds to the

well-recognized GMH of the preterm, yet occurring in utero (Kirton and deVeber, 2009; Fluss et al., 2019).

Neonatal AIS is more common in boys than in girls (Golomb et al., 2004; Laugesaar et al., 2007; Grunt et al., 2015; Dunbar et al., 2020), presumed PVI (Ilves et al., 2016) and also presumed AIS is more common in girls (Laugesaar et al., 2007). Girls are more disposed to thrombus formation and embolic risk leading to stroke (Parsa and Robert, 2013). Female predominance in presumed perinatal stroke, both AIS and PVI, could also be explained by increased mortality among male fetuses with stroke (Ilves et al., 2016).

PVI has not been associated with any intrapartum complications or a difficult transition (Ilves et al., 2016). On the contrary, acute intranatal conditions occur more often in children with presumed AIS compared to PVI. Most presumed AIS cases probably occur around birth with mild neonatal symptoms, of which at least some cases can be missed during the neonatal period (Ilves et al., 2016; Fluss et al., 2019). Acute perinatal factors were rare in children with presumed PVI (17%) compared to children with presumed AIS (66%) (Kirton et al., 2010). The proportion of deliveries by any caesarean section (42%) was greater than the corresponding typical rates in Canada and was similar in both AIS and PVI groups (Kirton et al., 2010). The proportions of emergency caesarean sections was not different between the groups (17% in AIS group and 9% in PVI group, p=0.41) (Kirton et al., 2010).

Prothrombotic risk factors, including protein C and S, antithrombin III, lipoprotein (a), homocysteine, and factor VIII levels; anticardiolipin antibodies and lupus anticoagulant; methylenetetrahydrofolate reductase (MTHFR) mutations, factor V Leiden, and prothrombin G20210A mutations, were investigated in children with presumed perinatal stroke (AIS in 60% and PVI in 40%). At least one prothrombotic risk factor was present in 69% of the cases, two in 17% of the cases, and three or more in 8.5% of the cases (Kocaman and Yilmaz, 2012). According to that study, most of the patients with presumed perinatal stroke might have had one or more prothrombotic risk factors, as well as certain prenatal risk factors including intrauterine growth retardation, twin gestation and preeclampsia, which might be associated with presumed perinatal stroke (Kocaman and Yilmaz, 2012). However, in contrast to earlier findings (Kocaman and Yilmaz, 2012; Suppiej et al., 2008), a recent report showed that previously thought hemophilia risk is not increased in children with perinatal stroke (Curtis et al., 2017). Genetic disorders of vascular integrity such as *COL4A1* pathogenic variants might predispose to GMH. This issue has not been well studied in PVI, with main focus being on rehabilitation (Dunbar and Kirton, 2019).

The above data demonstrate differences in the etiology of these stroke types and therefore the risk factors for presumed AIS should not be extrapolated to presumed PVI.

4.2.4. Pregnancy, delivery and neonatal risk factors for PVHI in preterm infants

Studies of the etiology and pathogenesis of severe IVH/PVHI have revealed multiple maternal, pregnancy and neonatal risk factors, with low gestational age as the most important single independent risk factor (Ancel et al., 2005; Bassan et al., 2007; McCrea and Ment, 2008; eurUS.brain group et al., 2020).

The pathogenesis of IVH/PVHI in preterm born children is multifactorial with a combination of intravascular, vascular and extravascular factors in patients with PVHI ("Volpe's Neurology of the Newborn – 6th Edition," n.d.). However, some authors have suggested that IVH in preterm neonates is a much more complex disorder where both fetal and postnatal environments as well as the genome play a role (Ment et al., 2014). Besides changes in the cerebral blood flow, also inflammation, coagulation and vascular factors can be essential (Ment et al., 2014).

Intravascular factors for development of GMH/IVH are related to regulation of the blood flow, blood pressure and volume, which can change circulation in the microvasculature of the germinal matrix ("Volpe's Neurology of the Newborn – 6th Edition," n.d.). Hypercarbia, decreased hematocrit, or decreased blood glycose level may contribute to elevations in the cerebral blood flow in premature infants, which can lead to IVH ("Volpe's Neurology of the Newborn – 6th Edition," n.d.). It has been shown that 87 of 90 infants with birth weight <1500 g have the pressure passive state for 20 to 50% of the time during the first five days of life (Soul et al., 2007). Several factors can lead to an increase in the cerebral blood flow in preterm children in the case of pressure passive circulation, including a sudden increase in systemic blood pressure, postnatal status, caretaking procedures like tracheal suctioning, systemic complications (like pneumothorax), ligation of the patent arterial duct, rapid volume expansion, seizures (Perlman and Volpe, 1983; Soul et al., 2007; "Volpe's Neurology of the Newborn – 6th Edition," n.d.). Fluctuations in the systemic and cerebral blood flow can be related to mechanic ventilation and respiratory distress syndrome, as well as other factors increasing the cerebral blood flow (Perlman and Volpe, 1983; "Volpe's Neurology of the Newborn – 6th Edition," n.d.). However, a decrease in the cerebral blood flow can also cause IVH by damaging the vasculature during hypoperfusion and disruption of vessels during reperfusion ("Volpe's Neurology of the Newborn – 6th Edition," n.d.).

Disturbances of platelet function and coagulation function may contribute to the pathogenesis of IVH, however, there exists disagreement regarding the causality of IVH ("Volpe's Neurology of the Newborn – 6th Edition," n.d.).

Vascular factors for development of IVH include the germinal matrix vessels themselves, which are fragile in nature: composed of simple endothelial-lined vessels, which are in progress of remodelation and involution and therefore more susceptible to rupture. Furthermore, the vessels are vulnerable to hypoxic ischemic injury as, like other brain capillaries, they appear to have high requirement for oxidative metabolism and contain more mitochondria compared to the

systemic capillary endothelial cells (Oldendorf et al., 1977; "Volpe's Neurology of the Newborn – 6th Edition," n.d.). Extravascular factors influencing the development of IVH include the deficient extravascular support of mesenchymal and glial elements to the vessels of the germinal matrix, which are large endothelial-lined capillaries (Gould and Howard, 1987).

Furthermore, an excessive amount in fibrinolytic activity has been found in the periventricular, germinal matrix region (Takashima et al., 1979; Gilles et al., 2009). This is needed for remodeling of the germinal matrix, but allows also small capillary hemorrhages to become large lesions ("Volpe's Neurology of the Newborn – 6th Edition," n.d.).

Perinatal asphyxia increases the likelihood of IVH and there exists strong association of the lesion with fetal distress, need for emergency caesarean section, low Apgar scores and need for respiratory resuscitation (Bassan et al., 2006b). Although not necessarily, the infant with prior perinatal asphyxia has an increased risk for development of IVH which tends to be large ("Volpe's Neurology of the Newborn – 6th Edition," n.d.).

A recent metaanalysis showed association between PVHI and emergency caesarean section in 30%, patent arterial duct in 19%, thrombocytopenia in 18%, pneumothorax in 12%, seizures in 11%, and intrauterine growth restriction and/or preeclampsia in 8% of cases (Roy et al., 2022). Prolonged prelabor rupture of the membranes and birth distress may be associated with postnatal onset of certain brain lesions, or may make the brain more vulnerable to subsequent insults in the neonatal period (de Vries et al., 1998). An omnious cardiotachogram was significantly more common among children with antenatal development of PVHI ($p=0.01$) in a prospective study with 1332 infants born before GW 34, with 24 cases with hemorrhagic and/or ischemic lesion, where the onset of development of PVHI was considered to be antenatal (de Vries et al., 1998).

Both gender and twin studies support the hypothesis that IVH is a more complex disorder than a mere postnatal vascular process. Preterm males are more likely than females to experience severe IVH (Kent et al., 2012). Studying 450 twin pairs, Bhandari and coworkers reported that 41.3% of the variance in IVH risk is attributable to familial and environmental factors (Bhandari et al., 2006).

Furthermore, although maternal transport, antenatal steroid administration and improved resuscitation techniques have become the standard of care in neonatal tertiary care units worldwide, the incidence of severe IVH (grade III IVH and PVHI) demonstrates a variable but persistently high burden for very low birth weight infants (Stoll et al., 2010; Handley et al., 2018). A recent study in California showed a decrease in severe IVH from 9.7 % in 2005 to 5.9% in 2015 among children born at GW <32 (Handley et al., 2018). Antenatal steroid exposure was the only factor associated with decreased odds to develop severe IVH for all gestational age subgroups and need for delivery room intubation was associated with increased odds to have severe IVH in a study with infants born at GW 22 to GW <32 (Handley et al., 2018).

The incidence of IVH is adversely related to gestational age, however, also transition to extra-uterine life and/or triggers to which the neonates are exposed to during birth contribute to hemorrhage, and both hypoxemia and inflammation have been implicated in severe IVH of the prematurely born child (Ment et al., 2014). It has been suggested earlier that not only environmental factors during pregnancy, distress at birth, or fluctuations in the cerebral blood flow, but also genetic factors may be important in development of severe intraventricular hemorrhage and PVHI in fetuses and neonates (Ment et al., 2014, 2015).

One of the reasons why the incidence of IVH/PVI is still high can be that less attention has been paid to prenatal risk factors and genetic causes leading to PVHI.

4.2.5. Infections

One of the major insults during pregnancy is intrauterine infection. Fetal development occurs in the sterile amniotic cavity, although at least 25% of all preterm births occur in mothers with microbial invasion of the amniotic cavity (Romero et al., 2007). The human fetus is capable of cellular and humoral inflammatory response from the mid-trimester of pregnancy. The onset of premature labor in the case of infection is mediated by pro-inflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor alpha (TNF-α). These cytokines are produced by intrauterine tissues in response to microbial products and can stimulate prostaglandin production (Romero et al., 2007). Fetal inflammatory response has been defined by elevated concentrations of inflammation-related proteins in umbilical cord blood and by the histologic evidence of inflammation in the umbilical cord vessels. It occurs in a fraction of fetuses exposed to microorganisms in utero, and is associated with the onset of preterm labor, as well as with multisystem organ involvement (Romero et al., 2007).

Infection hazards during pregnancy have been reported earlier. Maternal infection can lead to preterm birth (Farkash et al., 2012). Furthermore, subclinical infection is a major cause of preterm birth, especially before GW 30 (Gibbs, 2001). The outcome of preterm birth has a direct consequence, as prematurity is a risk factor for a range of neurologic disorders leading to visual, auditory, motor and cognitive impairments (Platt, 2014; Ream and Lehwald, 2018).

An increased risk for severe GMH and poor neurodevelopmental outcome in preterm infants exposed to antenatal infection has been noted by several authors (Gagliardi et al., 2013; Abehsera et al., 2014). Chorioamniotis among other maternal infections can initiate a cascade of antenatal fetal inflammatory response with injury to the developing brain and other fetal organs as an end result (Abehsera et al., 2014). Histological chorioamnionitis in preterm birth has been associated with IVH and periventricular leukomalacia, leading to cerebral palsy and higher relative speech delay and hearing loss (Suppiej et al., 2009). A metaanalysis showed that clinical and histological chorioamnionitis were

significantly associated with cerebral palsy (relative risk: 1.9; [95% CI, 1.4–2.5]; 1.6; [95% CI, 0.9–2.7], respectively) in preterm infants (Wu and Colford, 2000).

While infection during pregnancy has been studied relatively well, then studies of long term outcome in term born children after the mothers' bacterial infection during pregnancy are still insufficient and scarce. In a population based cohort study, the third-trimester diagnosis of any hospital-reported maternal infection revealed association with cerebral palsy in term born children (hazard ratio 1.8, [95% CI 1.2–2.8] (Miller et al., 2013). Significant association of clinical and histological chorioamnionitis with cerebral palsy was found in meta-analyses (Wu and Colford, 2000; Shatrov et al., 2010). Positive association was found between clinical chorioamnionitis and cerebral palsy in term born children (relative risk, 4.7; [95% CI, 1.3–16.2] (Wu and Colford, 2000).

Persisting relationship between umbilical cord inflammation and elevated concentrations of inflammation-related proteins in the blood on postnatal day 7 suggests the existence of phenomena that contribute to a reinforcement loop and thereby sustained systemic inflammation in neonates (Leviton et al., 2011).

4.2.6. Genetic risk factors

In some PVHI/PVI cases genetic predisposition like connective tissue disorders might be the leading cause of developing IVH (Yoneda et al., 2012, 2013; Ment et al., 2014; Zagaglia et al., 2018; Maurice et al., 2021).

Candidate gene studies implicate the inflammatory, coagulation and vascular pathways, and recent data suggest that there may be differences in the time of hemorrhage (Harteman et al., 2012; Ment et al., 2014, 2015). In earlier studies the potential risk factors for PVHI were genetic mutations of MTHFR gene in 88%, factor V Leiden gene in 41%, COL4A1 gene in 12% and prothrombin gene in 6% (Harteman et al., 2012). However, MTHFR was disproven later (Hickey et al., 2013), as was also disproven association between neonatal stroke and thrombophilia (Curtis et al., 2017).

4.2.6.1. Genes associated with collagenopathy and vasculopathy

Collagen normally consists of the triple helix whose configuration can be disrupted if the aminoacid Glycine is replaced because of mutations in the *COL4A1* gene, which is the underlying mechanism of most pathogenic variants.

Abundant collagen IV participates in formation of the basal membrane in a wide range of tissues, including vessel wall. *COL4A1* variants are found more often compared to *COL4A2* variants. An altered *COL4A1* gene compromises the structural integrity of the vascular basal membrane by causing capillary basement membranes to interrupt, and accounting for both normal and markedly increased thickness of the membrane (Knaap et al., 2006). This damage to small capillaries causes microangiopathy with variable clinical severity from asymptomatic to fatal cases including development of porencephaly and/or schizen-

cephaly (Vilain et al., 2002; Knaap et al., 2006; Verbeek et al., 2012; Yoneda et al., 2013; Meuwissen et al., 2015; Zagaglia et al., 2018; Hausman-Kedem et al., 2021a; Maurice et al., 2021).

Type IV collagen variants, being abundant in different tissues, are responsible for a wide spectrum of phenotypes involving multiple organ systems, with brain abnormalities and neurologic sequelae including stroke being more prevalent in children with collagenopathies (Yoneda et al., 2013; Meuwissen et al., 2015; Zagaglia et al., 2018). Furthermore, an analysis of 15 pedigrees with *COL4A1* variants revealed worsening of severity of the clinical phenotype in succeeding generations, especially when maternally inherited (Zagaglia et al., 2018).

4.2.6.1.1. Risk factors in children with collagenopathy and vasculopathy

Earlier, no pregnancy or delivery risk factors were found to distinguish children with PVHI/PVI and collagenopathy from those without an identified genetic cause of PVHI/PVI (Zagaglia et al., 2018). None of the mothers with pregnancy complications carried the mutation of collagenopathy detected in the affected child (Zagaglia et al., 2018).

Head circumference at birth indicated microcephaly in 15/20 children with COL4A1 variants (Zagaglia et al., 2018). Perinatal stroke should be one of the differential diagnoses in children with microcephaly at birth (Yoneda et al., 2013; Zagaglia et al., 2018).

4.2.6.1.2. Genetic risk factors and time of development of PVHI/PVI

In fetuses with COL4A1/A2 variants the median age at the diagnosis of fetal hemorrhage was GW 24 (22–26) and in fetuses without genetic changes GW 32 (29.5–34.5) (Maurice et al., 2021). PVHI/PVI usually occurs between GW 24 and 34 when the germinal matrix is the most prominent and fragile (Dunbar and Kirton, 2019; Fluss et al., 2019; eurUS.brain group et al., 2020). It can be presumed that the germinal matrix is more likely to bleed due to disruption of the immature fragile vessels weakened even further by pathogenic variants in COL4A1/A2 or COL5A1.

4.2.6.1.3. MRI findings in children with collagenopathy

Children with *COL4A1/A2* variants show a wide spectrum of severity of MRI findings (Knaap et al., 2006; Yoneda et al., 2013; Meuwissen et al., 2015; Zagaglia et al., 2018; Maurice et al., 2021; Itai et al., 2021). Collagenopathies cause microangiopathy, with variable clinical severity, from asymptomatic to fatal cases (Vilain et al., 2002; Knaap et al., 2006; Verbeek et al., 2012; Yoneda et al., 2013; Meuwissen et al., 2015; Zagaglia et al., 2018; Maurice et al., 2021; Hausman-Kedem et al., 2021a).

Microbleeds, cerebral white matter abnormalities and lacunar infarcts in children with collagenopathies suggest a pathogenesis similar to that of micro-

angiopathy (Knaap et al., 2006; Meuwissen et al., 2015; Zagaglia et al., 2018; Maurice et al., 2021; Hausman-Kedem et al., 2021a).

Malformations of cortical development, including schizencephaly, polymicrogyria, focal cortical dysplasia, and nodular heterotopia, were noted in children with collagenopathy and these findings were always associated with signs of white matter vascular insult (i.e., periventricular leukoencephalopathy, ventricular dysmorphisms, or white matter thinning) (Zagaglia et al., 2018).

Children with PVI/PVHI usually have single, asymmetric periventricular white matter damage, persistent over time (Bassan et al., 2006a, 2007; eurUS.brain group et al., 2020). However, in children with collagenopathies, the porencephalic enlargement of the lateral ventricles and/or periventricular white matter damage can be unilateral or bilateral (Knaap et al., 2006; Yoneda et al., 2013; Maurice et al., 2021). Children with collagenopathies develop severe porencephaly involving several lobes, or multifocal porencephalic brain damage with irregular borders which can indicate different ages of development of stroke (Maurice et al., 2021). Severe and/or multifocal ischemic/hemorrhagic cerebral lesions can be of different ages of stroke and associated with schizencephaly and porencephaly (Maurice et al., 2021). Signs indicating microangiopathy in the case of collagenopathy include extensive bilateral diffuse white matter T2 weighted signal hyperintensities and extensive bilateral white matter loss and lacunes on MRI (Lanfranconi and Markus, 2010; Zagaglia et al., 2018). Calcifications have been reported on computed tomography (CT) examination in children with collagenopathy (Yoneda et al., 2013; Itai et al., 2021; Maurice et al., 2021).

4.2.6.1.4. Outcome in children with collagenopathy

Children with collagenopathies show a wide spectrum of phenotypes causing congenital hemiparesis, epilepsy and intellectual disability (Meuwissen et al., 2015; Zagaglia et al., 2018; Hausman-Kedem et al., 2021a; Itai et al., 2021). Mutations associated with epilepsy were spread across *COL4A1*, while no clear genotype-phenotype correlation was found (Zagaglia et al., 2018). Earlier, epilepsy has been diagnosed in 55/123 patients with *COL4A1/2* pathogenic variants (Zagaglia et al., 2018). Childhood-onset epilepsy, frequently complicated by status epilepticus and resistance to antiepileptic drugs, was the most common phenotype in children with collagenopathy. In 46.4% of patients with focal seizures, porencephalic cysts on brain MRI were localized in the same area as focal epileptiform discharges (Zagaglia et al., 2018).

Outcome in patients with collagenopathies can worsen due to spontaneous intracranial macrohemorrhages later in life (Knaap et al., 2006). Collagenopathies are recognized also in the adult population as a monogenic cause of small vessel disease (Lanfranconi and Markus, 2010).

4.2.6.2. Inherited Coagulopathies

Gene variants leading to coagulation abnormalities have been considered as a possible cause of IVH, because of both the pathophysiology of hemorrhage and their putative role in perinatal stroke ("Volpe's Neurology of the Newborn – 6th Edition," n.d.).

There is still uncertainty regarding the role of thrombophilia in the pathogenesis of perinatal stroke. In preterm born children the most widely studied gene variants include the factor V Leiden (F5) variant, MTHFR gene polymorphisms and the prothrombin 20210G>A variant (F2) (Ment et al., 2014). However, MTHFR as the cause of stroke has been disproven (Hickey et al., 2013). A study of preterm infants with an atypical presentation of PVHI suggested testing for thrombophilia, in particular for the factor V Leiden and prothrombin gene variants found in 7/17 cases; however, also *COL4A1* gene variants were found in 2/17 cases (Harteman et al., 2012).

Coagulation abnormalities as a possible cause of perinatal stroke have been suggested by some authors (Mineyko and Kirton, 2011; Cavaliere et al., 2021), while others have found the thrombophilia risk not to be increased in perinatal stroke, including presumed antenatal PVI and AIS in term born children versus control (Laugesaar et al., 2010; Curtis et al., 2017). Earlier proposed thrombocytopenia and maternal and fetal clotting disorders and trauma are relatively rare, and the significance of endothelial dysfunction rather than thrombocytopenia has been emphasized (Kirkham et al., 2018).

The caucasian population shows the highest prevalence of the prothrombin (F2) 20210G>A [rs1799963G>A] genetic variant and the factor V Leiden mutation, which both predispose to inherited thrombophilia (Harteman et al., 2012). The prevalence of the carriers in Estonia was 3.3% for the prothrombin common variant and 3% for the Factor V Leiden variant among 400 controls (Laugesaar et al., 2010). Cavaliere and coworkers found two case reports describing factor V deficiency, but none describing the prothrombin 20210G>A variant in a review article on the genetic profiling of antenatal intracranial hemorrhage (Cavaliere et al., 2021).

However, genetic thrombophilia may predispose patients to onset of SVT after birth, at least in preterm infants, but also during infancy and childhood (Laugesaar et al., 2010; Cavaliere et al., 2021). Likewise, genetic thrombophilia may increase the risk of early onset ischemic stroke in young adults (Jiang et al., 2014). Although the dominating proposed mechanism of PVHI/PVI is a complication of GMH, medullary vein thrombosis would have an identical appearance and may be a relevant finding in children with prothrombotic gene variants, particularly in symptomatic term neonates.

4.2.6.3. Other genes associated with stroke

There are other gene variants that can lead to microangiopathy and stroke or stroke-like episodes, which can be familial. The most common mitochondrial variant m.3243A>G in *MT-TL1* is known to cause the MELAS (Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like Episodes) syndrome, which leads to microvascular blood perfusion damage and stroke-like episodes at the age under 40 years with serious long-term consequences (Cheng et al., 2022). The patients can have hearing loss, diabetes and migraine. However, the causality of mitochondrial DNA variation in the MELAS syndrome in relation to perinatal stroke is uncertain.

A pathogenic change in *NOTCH3* leads to CADASIL (Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). Changes in *NOTCH3* lead to cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, including white matter hyperintensities, lacunar infarcts and microbleeds (Stojanov et al., 2015). The prenatal diagnosis of the *NOTCH3* variant has been reported in a fetus whose father had this known variant (Milunsky et al., 2005); however, this finding has not been reported for children with perinatal stroke. The mean age of symptom onset in these patients is known to be before 45 years, with attacks of migraine with aura occurring early in life, however MRI can detect changes even before typical clinical symptoms (Chabriat et al., 1995).

4.3. Outcome of perinatal stroke

4.3.1. Outcome in term born children with presumed antenatal PVI

Outcome in children with perinatal stroke is diverse. Perinatal stroke causes damage to the developing sensorimotor system, including the motor and somatosensory tracts, which leads to contralesional spastic hemiplegia (Kirton and deVeber, 2009, 2013; Lõo et al., 2018; Craig et al., 2019). The vascular stroke type is important when evaluating prognosis for children with perinatal stroke, as cognitive deficit, language delay and behavioral problems vary between the different types of perinatal stroke (Laugesaar et al., 2018; Wagenaar et al., 2018; Ilves et al., 2022a, 2022b). In a study combining both AIS and PVI, children with perinatal stroke showed lower memory, verbal and visual learning performances compared to healthy controls (Virani et al., 2022).

Both children with AIS and PVI have significantly lower cognitive and language scores compared to controls (Ilves et al., 2022b). Children with AIS seem to have a high risk of complex cognitive and behavioral disorders compared to children with PVI (Kirton et al., 2008; Lõo et al., 2018). Large periventricular injury in children with PVI leads to lateralization of the language

center to the right as is seen in neonates with AIS who have poor outcome of language function (Ilves et al., 2022b).

The risk for development of epilepsy varies among different vascular perinatal stroke types (Laugesaar et al., 2018). Epilepsy risk depends largely on the vascular type and location of stroke (Wusthoff et al., 2011; Laugesaar et al., 2018) and the risk of epilepsy remains elevated even 20 years after stroke (Sundelin et al., 2021). Epilepsy risk was significantly higher among children with neonatal and presumed antenatal AIS (61% after 8.6 years of age) compared to children with presumed antenatal PVI (6%), however, children with PVI tended to suffer from severe and drug-resistant epilepsy (Laugesaar et al., 2018).

4.3.2. Outcome in preterm born children with PVHI

The rate of prematurity has been high, accounting for 12–13% of births in the United States and as many as 64–75% of infant deaths, and 42–47% of cerebral palsy cases (Allen, 2008). Globally there is no measurable change in preterm birth rates from 2010 to 2020 and approximately 15% of all preterm births occur at less than GW 32 (Ohuma et al., 2023).

The advances in obstetric and neonatal medical care and assisted reproductive technology during the last five decades have increased the rate of preterm births and decreased preterm infant mortality rates. Infant mortality was rapidly and significantly reduced among children born at GW <32 with antenatal steroid administered before delivery in multicenter studies both in Europe and California (Norman et al., 2017; Handley et al., 2018). In Estonia the survival of very preterm children born at 22–31 GW increased from 78% in 2002–2003 to 85% in 2007–2008 ($p=0.041$) (Toome et al., 2012) and from 2011–2012 survival for this cohort was above 90% (Zeitlin et al., 2016). However, among extremly premature infants of GW 22– 25 mortality rate has been as high as 50% and half of the surviving children have severe neurodevelopmental disabilities (Jarjour, 2015).

A recent multicenter study of neurodevelopmental outcome in children with median gestational age of GW 26.6 with PVHI showed that 40% of the children died in the neonatal period and the rate of cerebral palsy among the survivors was 42%; the composite outcome of death or severe neurodevelopmental disability was 58% (Cizmeci et al., 2020). Two thirds of PVHI survivors develop significant cognitive and/or motor deficit (Bassan et al., 2007; Jarjour, 2015). In a recent systematic review the mortality rate of preterm infants between GW 23–36 with stroke was found to be 33% and the rate of cerebral palsy in children with PVHI was 36% (Roy et al., 2022).

Large size of PVHI is associated with death or severe spastic contralesional hemiplegia (Dudink et al., 2008; Cizmeci et al., 2020). Parietal location of PVHI was connected with higher mortality and PVHI in the temporal and frontal lobes carried the risk of cognitive, behavioral and visual problems (Soltirovska Salamon et al., 2014). Although posthemorrhagic ventricular dilation is

more frequently observed after severe grade of IVH and PVHI, it may complicate each grade of IVH if bleeding has extended into the ventricular lumen (eurUS.brain group et al., 2020). Posthemorrhagic ventricular dilation in 36% of the patients with PVHI was an independent risk factor for poorer cognitive and motor outcomes ($p<0.001$ for both) (Cizmeci et al., 2020).

4.4. Summary of the literature review

Perinatal stroke is a rare disease with poor outcome. While preterm infants with lower gestational age and higher PVHI risk survive more often owing to improved intensive care, then the number of preterm born neonates with severe IVH/PVHI is still high. Better recognition of children with presumed perinatal stroke has increased also the number of patients with presumed antenatal PVI.

PVI in term born children and PVHI in preterm born children may lead to lifelong, often combined, deficit of motor, cognitive and language functions. The child with stroke has to live many years with the disability. Rehabilitation can mitigate stroke sequelae, but it can not eliminate the irreversable brain damage. The best option of alleviating the burden of perinatal stroke is to avoid it by eliminating its causative factors, but regrettably, these are not yet clear.

Severe IVH /PVHI are multifactorial diseases. However, changes in the treatment strategies of very preterm infants, including transportation of risk pregnancies to higher care centers, use of new medications like prenatal glycocorticoids, magnesium sulfate and postnatal surfactant, as well as changes in intensive care, with the aim to avoid blood flow fluctuations, result in higher survival of very premature infants who still present a challenge, regarding severe IVH and PVHI.

Earlier studies have shown that some preterm neonates have PVHI already at birth, suggesting that the antenatal mechanism may be more important than previously thought. Evaluation of prenatal risk factors in comparison to control may add further information, particularly regarding children with prenatal development of severe IVH/PVHI and those with presumed antenatal PVI. Small cohort studies have not found clear evidence about the etiology and exact time of development of IVH/PVHI during pregnancy or about its exact mechanism in term born children with presumed antenatal stroke who develop lifelong disability. Genetic evaluation with inclusion of genetic tests for collagenopathies in children with PVHI/PVI has not yet been systematically implemented.

5. AIMS OF THE STUDY

The general aim of the study was to investigate the etiology and timing of risk factors for presumed antenatal PVI and PVHI. We hypothesized that PVHI/PVI diagnosed at different time points shows similarities in risk factors and time of their occurrence.

The specific aims and the hypotheses:

1. The aim of the study was to compare term or near-term born children (≥36 GW) with presumed antenatal PVI, preterm-born children (<36 GW) with PVHI, and consecutively born unaffected control infants (≥36 GW), to identify the prevalence of pregnancy and delivery associated risk factors, and gestational age at their occurrence. (Substudy I)

   The hypothesis was that pregnancy and delivery associated risk factors for presumed antenatal PVI and gestational age at their appearance are similar in term born children with presumed antenatal PVI and in preterm children with PVHI, while PVI and PVHI are diagnosed at different ages. (Substudy I)
2. The aim of the study was to compare pregnancy and delivery associated risk factors in preterm neonates with antenatal and neonatal onset of severe IVH/PVHI. (Substudy II)

   The hypothesis was that the profile of pregnancy and delivery associated risk factors is different in preterm neonates with antenatal compared to neonatal onset of severe IVH/PVHI. (Substudy II)
3. The aim of the study was to find the prevalence of the genetic variants previously associated with stroke in children with PVHI/PVI using genome-wide analysis. (Substudy III)

   The hypothesis was that children with PVHI/PVI show high prevalence of collagenopathies, as has been found earlier in fetuses and in children with porencephaly. (Substudy III)

6. SUBJECTS AND METHODS

6.1. Study population

Term born children with antenatal PVI, presumed antenatal PVI and preterm born children with PVHI were identified from the Estonian Pediatric Stroke Database (EPSD) using the substudy specific criteria for patient selection.

6.1.1. Patient collection from the Estonian Pediatric Stroke Database

EPSD for term born children was created for an epidemiological study and subjects were retrospectively included from 1994 to 2003. From 2004 the subjects treated and/or investigated at Tartu University Hospital were included prospectively (Laugesaar et al., 2007; Ilves et al., 2016; Laugesaar et al., 2018; Lõo et al., 2018).

Term born children are included in EPSD if they have been diagnosed with perinatal stroke and have undergone confirmative radiological investigation: CT or MRI. The diagnosis of perinatal stroke is based on the independent evaluation of radiological images by three pediatric neuroradiologists who are blinded to the findings of clinical outcome. The vascular type of stroke in children with EPSD is classified according to the criteria outlined by Kirton et al. (2008) and modified by Ilves and colleagues (Ilves et al., 2014, 2016). The patient is included in EPSD, following a consensus meeting of neuroradiologists and neurologists, concerning the diagnosis of perinatal stroke and its vascular type.

Preterm born children with severe IVH/PVHI in EPSD were identified from the clinical database of the third level regional Tartu University Hospital where about one third of the neonatal cases in the country were treated in 1994–2019. The medical records of the neonatal intensive care unit of the hospital were searched for children with the diagnosis of severe grade IVH (grade III or IV) or PVHI (Bassan et al., 2006a; Handley et al., 2018) treated at the hospital between 1994 and 2019. Also the hospital's electronic database from 2008 was searched for diagnosis codes P 52.2., .3, .4, .6, .9 according to the international classification of diseases 10th revision, ("ICD-10 Version:2019," n.d.). Patients with grade III IVH were included in the initial search to find all possible patients with PVHI confirmed later by MRI or autopsy. After the initial search and radiologically and clinically confirmed diagnosis the patients were included in EPSD.

GMH-IVH was graded based on the findings of cerebral US as follows: grade I – germinal matrix hemorrhage; grade II – intraventricular blood without distension of the ventricular system; grade III – blood filling >50% of the ventricle and distending the ventricular system; and grade IV – hemorrhage involving periventricular parenchymal tissue, also known as PVHI (Papile et al., 1978; Szymonowicz and Yu, 1984; Elchalal et al., 2005; "Volpe's Neurology of the Newborn – 6th Edition," n.d.; eurUS.brain group et al., 2020). A patient with bleeds of various severity was assigned a grade based on the most severe documented bleeding or stroke.

PVHI was defined as a parenchymal increase in echogenicity in the periventricular area, which can be fan- or globular-shaped and separate or partially communicating with the ventricle, the area progressing into a small periventricular cystic lesion or a larger porencephalic cyst with enlargement of the lateral ventricle on cerebral US (Rademaker et al., 1994; de Vries et al., 2001; Elchalal et al., 2005; Dudink et al., 2008).

All cerebral US were performed by pediatric radiologists during the entire study period. Initially, US was done on the first day after the patient was stabilized, the next imaging was done on the 2nd-3rd day and 5th-7th day and further once a week until discharge from the hospital. US was promptly repeated, if the status of the patient deteriorated and severe IVH/PVHI was clinically suspected.

The diagnosis of PVHI was confirmed by MRI performed around the postmenstrual age of GW 40 or later during infancy or at autopsy. In general, the neuroradiological definition of PVHI/PVI based on MRI was: mostly unilateral medullary venous territory infarction in the periventricular area, with porencephalic enlargement of the lateral ventricle and/or periventricular gliosis and/or smaller periventricular cystic changes or larger porencephalic cyst; and hemosiderin deposits detected in the region of the germinal matrix, lateral ventricle and/or in the periventricular area (Takanashi et al., 2003; Elchalal et al., 2005; Kirton et al., 2008, 2010; Kirton and Wei, 2010).

The exclusion criteria for the EPSD are 1) absence of confirmative cranial imaging (MRI or CT) or autopsy findings; 2) diseases other than stroke involving the central nervous system, as suggested on the basis of neuroimaging and clinical findings like a) severe birth asphyxia including watershed infarction or periventricular leucomalacia; b) infectious diseases including meningoencephalitis without infarction; c), metabolic and mitochondrial disturbances and diseases including hypoglycemia and kernicterus leading to brain damage; d) tumors; e) other cortical malformations or congenital anomalies not associated with perinatal stroke.

For this study, only neonates with PVHI/PVI were retrieved from EPSD according to the inclusion criteria of the substudies. Neonates were excluded if they had at least one of the following: (1) acute or presumed AIS, (2) SVT, (3) parenchymal HS in locations other than the periventricular area, (4) other diseases, congenital anomalies or genetic diseases involving the central nervous system, (5) being second child of a twin pregnancy when both had severe IVH or PVHI.

6.1.2. Patient collection: Controls

The control groups for substudies I and II were formed of the cases in the observational pregnancy cohort of Happy Pregnancy Study (full name: Development of novel non-invasive biomarkers for fertility and healthy pregnancy, principal investigator Prof. Maris Laan, http://www.happypregnancy.ut.ee/en). The study comrises approximately 2/3 of all pregnant women receiving antenatal care and recruited prospectively during the first antenatal visit at the Women's Clinic of Tartu University Hospital (n=2359) in 2013–2015. As a

third level regional university hospital, Women's Clinic serves Eastern and Southern Estonia, where one third of the neonates of the country are born. The study specific cohort of mother-child pairs was retrieved for both substudies: term born children GW ≥36 and mothers (n=2334) for Substudy I and preterm born children before GW 35 (n=50) for Substudy II.

All participants of the study and the control groups were of white European ancestry and living in Estonia.

6.1.3. Inclusion criteria of Substudy I

Children born at GW ≥36 with antenatal PVI, presumed antenatal PVI and preterm born children GW <36 with PVHI from EPSD were included in the study group.

The control group for Substudy I consisted of mother-child pairs with deliveries at GW ≥36. Only the pregnancy and delivery data of the first twin was used in the case of twin pregnancies (n=25 in the control group). The final group comprised of 2168 term born children in the control group, 43 patients with presumed antenatal PVI, six with antenatal PVI and 86 with PVHI. The final control group (n=2,168) contained no cases of perinatal stroke.

The flow chart of the inclusion and exclusion criteria for PVHI/PVI and control patients is shown in Figure 3.

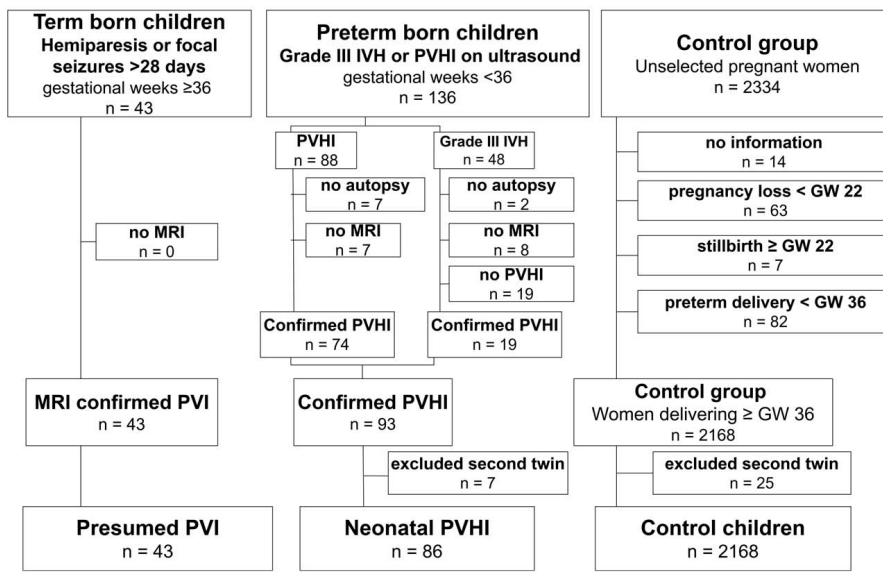

Figure 3. Flow chart of the study groups and the applied patient selection criteria (Substudy I). (GW, gestational weeks; IVH, intraventricular hemorrhage; MRI, magnetic resonance imaging; PVHI, periventricular hemorrhagic infarction; PVI, periventricular venous infarction). (Ilves et al., 2022, published with permission from Sage Journals).

6.1.4. Inclusion criteria of Substudy II

Preterm neonates GW <35 identified from EPSD were included if at least one of the following findings on the first cerebral US was present: (1) severe IVH (grade III or IV IVH) or PVHI, (2) residual intraventricular clots or strains, (3) periventricular cystic changes within the periventricular stroke area ≤ 3 days (de Vries et al., 1998; Bassan et al., 2006a; Handley et al., 2018; eurUS.brain group et al., 2020).

Most of the mother–child pairs were under observation and the children with IVH/PVHI were delivered at the third level university hospital. If needed, pregnant women with imminent preterm delivery were transported to the third level maternity hospital. However, 26 of 120 (22%) premature neonates with IVH/PVHI were born at county hospitals and transferred to a tertiary neonatal intensive care unit by a specialized team including an anesthesiologist from the neonatal intensive care unit.

Neuroradiologists, blinded to the clinical outcome of the patients, reviewed independently all radiological images. In all cases, cerebral US findings were available for review. MRI was available for review in 51/64 (80%) cases, where the patient survived and the parents gave consent for MRI investigation. Autopsy was available in 47/56 (84%) cases, including one patient (1.8%) with MRI image done prior to death. A case was reclassified as PVHI, if MRI (n=13) or autopsy (n=6) revealed also a periventricular infarction instead of only a grade III IVH detected on cerebral US.

Preterm neonates with severe IVH/PVHI were grouped based on the detection time of severe IVH/PVHI on cerebral US:

1) Antenatal onset group with (a) acute grade III IVH or PVHI visible on the initial cerebral US before 12 h of age or (b) periventricular cystic changes in the area of PVHI visible within 3 days of life, indicating antenatal development of PVHI (de Vries et al., 1998; eurUS.brain group et al., 2020).
2) Unknown time-onset group with grade III IVH or PVHI visible on the initial cerebral US performed later than 12 h of age, leaving the time of its development unknown.
3) Neonatal onset group with initial normal cerebral US or grades I-II GMH-IVH with later progression of hemorrhage to grade III IVH or PVHI.

The control group for Substudy II was based on the observational study of Happy Pregnancy cohort and mother-child pairs with deliveries occurring before GW 35. Of 2334 unselected pregnant women at the third level hospital, 51 had preterm delivery before GW 35. One mother-child pair was excluded since the child developed PVHI and was included in the study group.

The flow chart of the inclusion criteria for preterm born children with PVHI and control patients is shown in Figure 4.

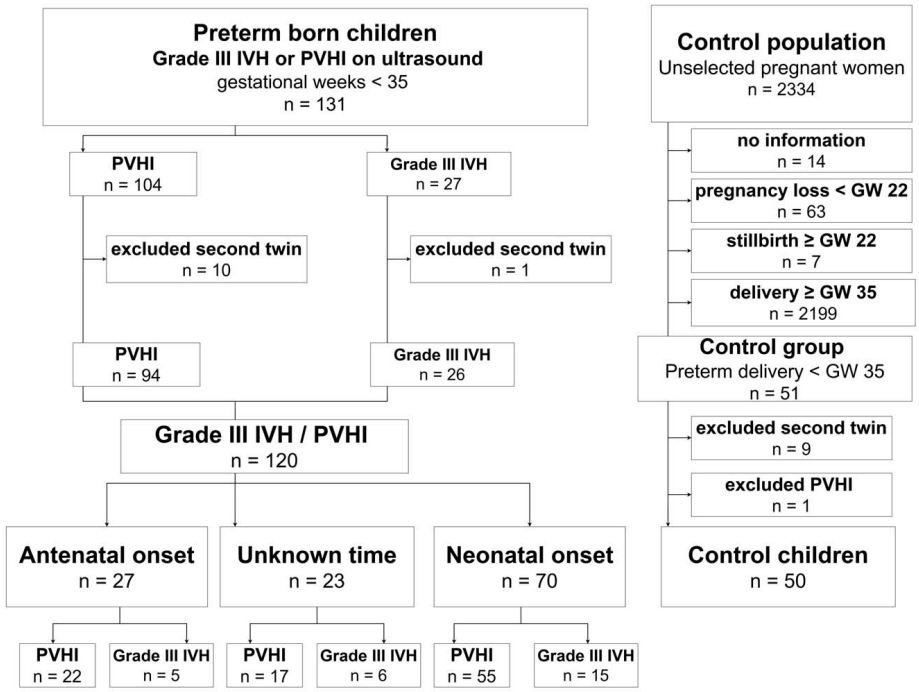

Figure 4. Flow chart of the study groups and the applied patient selection criteria (Substudy II). (GW, gestational weeks; IVH, intraventricular hemorrhage; PVHI, periventricular hemorrhagic infarction). (Ilves et al., 2024, published with permission from IOS Press).

6.1.5. Inclusion criteria of Substudy III

The data of term or near term born children (GW ≥36) with antenatal PVI (n=6), or with presumed antenatal PVI (n=43) were drawn from EPSD for 1994–2019.

Preterm born patients with PVHI from EPSD were included in the study group if they fulfilled all inclusion criteria a) gestational age ≤36 GW; b) severe grade of IVH (grade III or IV) or PVHI; c) treated at the university hospital between 2002 and 2019; d) the diagnosis of PVHI confirmed by autopsy or MRI, except for one preterm born child with PVHI (clearly visible on US), whose parents declined MRI, but agreed with genetic testing.

The final study group consisted of 39 preterm patients with PVHI, six antenatally diagnosed children with PVHI and 40 children with presumed antenatal PVI.

Control group for the genetic study. A database aggregating exome and genome data from large sequencing projects, the Genome Aggregation Database (gnomAD v3.1 spans 76,156 genomes), was used to check the variants and allele frequencies in the study group (Karczewski et al., 2020). In addition, the

sequencing data of approximately 5,000 exomes and genomes was sent to the gnomAD by the Estonian Biobank, these are listed in a separate section in gnomAD and can be considered as the healthy control group.

The flow chart of the inclusion criteria for the final study group of children with PVHI/PVI (n=85) is shown in Figure 5.

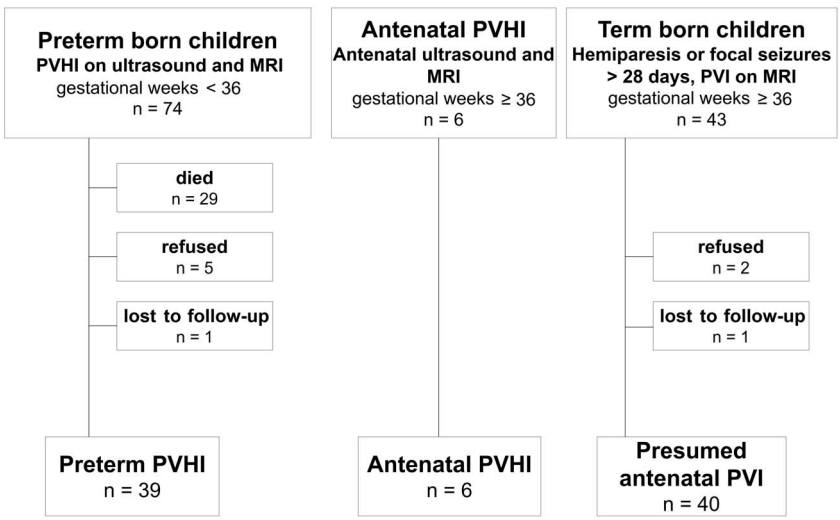

Figure 5. The flow chart of the inclusion criteria for children with PVHI/PVI for genetic testing. (GW, gestational weeks; IVH, intraventricular hemorrhage; MRI, magnetic resonance imaging; PVHI, periventricular hemorrhagic infarction; PVI, periventricular venous infarction). (Ilves et al., 2023, published with permission from Sage Journals).

6.2. Methods

6.2.1. Etiology assessment of stroke (Substudies I–III)

6.2.1.1. Perinatal risk factors

Clinical information on maternal, delivery, and neonatal data was obtained from paper- and electronic medical databases.

The data about pregnancy, delivery and neonatal condition were requested from three different tertiary maternity hospitals (one for the southern and eastern parts and two for the northern and western parts of the country), two central hospitals and eight county hospitals where the term born children with presumed antenatal PVI or antenatal PVHI or preterm children with PHVI had received antenatal care or where the children had been born. For all children with a respective pathology, the records of pregnancy and delivery, neonatal data, and records from the intensive care unit, when applicable, were reviewed. In the case of a twin pregnancy, with both children having stroke, EPSD con-

tains the data of risk factors during pregnancy and delivery. However, in this case, only the first child was included in our study in order not to overestimate the risk factors during pregnancy. The neonatal symptoms and outcome data for both children with PVHI/PVI were included.

For the prospectively collected control group, reproductive history, parental lifestyle, pregnancy risk factors and other epidemiological data were collected from three questionnaires completed at each trimester of pregnancy. Furthermore, the longitudinal clinical data about the pregnancy course and delivery outcome for these control patients were extracted from medical records.

Data on in- and out-patient antibacterial treatment during pregnancy was retrieved from the hospital's medical records (available for all study patients) and from the population based Digital Prescription Centre (available only for the control group) along with the exact diagnosis of infection according to the international classification of diseases 10th revision ("ICD-10 Version:2019," n.d.). Only the infections treated with antibiotics were included.

The clinical information included:

1) **risk factors present at the onset of index pregnancy**: maternal age, gravidity, parity, previous abortions, preterm delivery in reproductive history, threatened pregnancy loss GW <22 or imminent preterm delivery GW ≥22 during previous pregnancies, previous fetal and neonatal death, mode of conception (natural or in vitro fertilization), pregestational hypertension and diabetes mellitus, multiple gestation;
2) **risk factors during index pregnancy** with detailed information on gestational age at the onset of risk factor: attended antenatal care, threatened pregnancy loss GW <22, imminent preterm delivery GW ≥22, bacterial infections treated with antibiotics, smoking during pregnancy, alcohol abuse (consumption at least once a week), polyhydramnion, oligohydramnion, hypertensive disorders or diabetes mellitus during pregnancy, intrauterine growth retardation (<3 or <10 percentiles based on the national level of growth curves (Sildver et al., 2015)); anemia (hemoglobin <110 g/l in the first and third trimesters, <105 g/l in the second trimester of pregnancy);
3) **delivery data:** administration of antenatal steroids and/or magnesium sulphate, delivery institution (third level hospital or other), mode of delivery, labor augmentation with oxytocin, placental abruption, clinically and/or histologically confirmed chorioamnionitis, shoulder dystocia, signs of fetal distress during delivery, Apgar scores at the 1st and 5th minute after birth or need for resuscitation (ventilation with mask, intubation, cardiac massage and/or cardiotonic treatment);
4) **newborn data**: birth weight, head circumference, gestational age, sex
   The neonate was classified as small for gestational age when birth weight by gestational age- and sex-adjusted weight centiles at birth was <10 percentiles, and restricted growth when birth weight was <3 percentiles according to national normal values (Sildver et al., 2015);
   head circumference was similarly evaluated based on the Fenton growth charts (Fenton and Kim, 2013);

5) reports on placental histology;
6) signs of hypoxic-ischemic encephalopathy, and other neonatal neurological symptoms and electroencephalography findings;
7) neonatal outcome: survival or age at death during the first hospitalization;
8) cause of death: multiorgan failure, sepsis, cardiopulmonary failure based on autopsy reports and/or end of life decision;
9) autopsy reports.

Risk factors collected only for preterm born neonates with severe IVH/ PVHI

1) **SNAPPE II-** scoring system data for intensive care unit's patients within 24 h of birth including data about mean blood pressure, lowest temperatuure and serum pH, PaO_2/FiO_2 ratio, multiple seizures, urine output, APGAR score, birth weight, evaluation of small for gestational age (Rachuri et al., 2019);
2) **neonatal complications:** need for surfactant and intubation at birth, hypothermia on admission to the intensive care unit <34˚ C or <36˚ C, duration of artificial ventilation, early sepsis before 48 h, meningitis, stage of idiopathic respiratory distress syndrome, signs of pneumothorax, pulmonary hemorrhage, neonatal seizures, metabolic acidosis, necrotic enterocolitis with signs of peritonitis or perforation, operative or medical treatment of persistent arterial duct (excluding those who died within 3 days without treatment for persistent arterial duct), need for cardiotonic treatment (dopamine, dobutamine or adrenaline), bronchopulmonary dysplasia (excluding those who died within 7 days).

6.2.1.2. Clinical definitions of maternal diagnosis

Pyelonephritis was defined by the presence of a positive urine culture and at least one of the clinical findings: fever, and flank or back pain.

Appendicitis was confirmed when a patient needed surgical intervention.

The diagnosis of genitourinary infection (GTI) included also chlamydiosis, gonorrhea, and trichomoniasis.

The diagnosis of preeclampsia followed the international guidelines at the time of recruitment (ISSHP, 2014): co-occurence of a new-onset hypertension (HTN; ≥140/ ≥90 mmHg) after GW 20 and proteinuria or other signs of maternal organ dysfunction (Tranquilli et al., 2014). Gestational hypertension was diagnosed as new-onset hypertension after GW 20 without proteinuria or other maternal organ dysfunction (Tranquilli et al., 2014).

Polyhydramnion was diagnosed when the deepest amniotic fluid pool was >8 cm and oligohydramnion was diagnosed when it remained <2 cm on the prenatal US scan.

Clinical chorioamnionitis was diagnosed when: 1) mother had fever higher than 39.0 degrees of Celsius; 2) mother had fever between 38.0 – 39.0 degrees of Celsius and fetal tachycardia or maternal leucocytosis (>15,000 cells/mm3)

or purulent cervical drainage. Histologically confirmed chorioamnionitis was based on the findings of the placenta obtained by the pathologist.

Classification of infections during pregnancy on the basis of the international classification of diseases version-10 ("ICD-10 Version:2019," n.d.):

1. Diseases of the respiratory system: codes J+O99.5
2. Diseases of the digestive system codes: K+O99.6
   a. K00-K14 Diseases of the oral cavity and salivary glands
   b. K35-K38 Appendicitis
3. Diseases of the genitourinary tract: codes A+O+N except for O99.5 and O99.6)
   a. A: Certain infectious and parasitic diseases including urogenital chlamydia (A 560)
   b. O: Pregnancy, childbirth and puerperium including chorioamnionitis including asymptomatic bacteriuria (O23.0–O23.9)
   c. N: diseases of the genitourinary system
4. Other bacterial infection:
   a. H: diseases of the eye and the ear
   b. I: diseases of the circulatory system
   c. L: diseases of the skin and the subcutaneous system

Pregnant women were grouped as follows: (1) women with infections of the genitourinary tract, (2) women with other infections including infections of the respiratory and digestive systems and all other organs, (3) women without bacterial infections.

The time of infection was identified on the basis of hospital admission or the first diagnosis of infection for the PVI/PVHI group and on the basis of the first prescription redemption's time for the control group.

6.2.2. Genetic testing

For term born children with antenatal or presumed antenatal PVI from 1994–2019 and for preterm children with PVHI from 2002, genetic testing was suggested as part of a larger follow-up study of patients with perinatal stroke.

Next-generation sequencing techniques were used to analyse DNA extracted from leukocytes in blood samples. Agilent SureSelectXT Human All Exon V7 library capture followed by sequencing on the Illumina NovaSeq 6000 platform (Illumina Inc.) was used to sequence whole exomes in 60 individuals. 23 individuals were sequenced in a clinical setting on the Illumina NextSeq platform using the TruSight One Expanded panel covering 6,700 genes associated with Mendelian diseases. Further, single gene testing in a clinical setting was used in two patients and they had received genetic diagnoses before next-generation sequencing became available. Aligning of the raw sequencing reads to the hg19 reference genome was performed using the BWA MEM algorithm (Li and Durbin, 2009). Firstly, GATK HaplotypeCaller (Van der Auwera et al., 2013) was used to call individual variants, before GATK GenotypeGVCFs was employed for multi-sample joint aggregation and re-annotation. The High Per-

formance Computing Center of the University of Tartu "UT Rocket." ("EOSC-Nordic | UT Rocket," n.d.) was used for read alignment and variant calling. Hail 0.1 (Hail Team. Hail 0.1. https://github.com/hail-is/hail) was used to annotate the variant call set. The data was uploaded to Seqr platform (https://github.com/broadinstitute/seqr) and variants were collaboratively analysed.

The variant filtration strategy focused on rare variants in the coding regions and splice-sites predicted to affect protein function based on known inheritance patterns in two gene lists: a) genes associated with stroke or associated with stroke through vascular stability, b) genes associated with coagulopathies.

Stroke related genes: ABCC6, AC124319.1, ACTA2, ACVRL1, ADA2, ATP1A2, ATP1A3, ATP7A, BRCC3, CACNA1A, CBS, COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A1, COL5A2, ELN, ENG, FBN1, GLA, GUCY1A1, HTRA1, JAG1, MTCP1, NF1, NOTCH3, PHACTR1, PIK3CA, PRRT2, RASA1, SAMHD1, SCN1A, SLC2A10, SMAD3, SMAD4, TGFB2, TGFB3, TGFBR1, TGFBR2.

Coagulopathy genes: ABCG5, ABCG8, ACTB, ACTN1, ACVRL1, ADAMTS13, ANKRD26, ANO6, AP3B1, AP3D1, APOH, ARPC1B, BLOC1S3, BLOC1S6, CDC42, CHST14, COL1A1, COL2A1, COL5A1, COL5A2, CYCS, DIAPH1, DTNBP1, ENG, ETV6, F10, F11, F12, F13A1, F13B, F2, F5, F7, F8, F9, FERMT3, FGA, FGB, FGG, FLI1, FLNA, FYB1, GATA1, GBA, GFI1B, GGCX, GNE, GP1BA, GP1BB, GP6, GP9, HABP2, HOXA11, HPS1, HPS3, HPS4, HPS5, HPS6, HRG, IFNAR2, IKZF5, ITGA2B, ITGB3, KDSR, KLKB1, KNG1, LAT, LMAN1, LPA, LYST, MCFD2, MECOM, MPIG6B, MPL, MTHFR, MYH9, NBEA, NBEAL2, ORAI1, P2RY12, PIGA, PLA2G4A, PLAT, PLAU, PLG, PROC, PROCR, PROS1, PROZ, PTPN11, PTPRJ, RASGRP2, RBM8A, RNU4ATAC, RUNX1, SERPINA10, SERPINC1, SERPIND1, SERPINE1, SERPINF2, SLC35A1, SLC45A2, SLFN14, SMAD4, SRC, STIM1, STXBP2, TBXA2R, TBXAS1, TFPI, THBD, THPO, TPM4, TUBB1, VIPAS39, VKORC1, VPS33B, VWF, WAS.

Sanger sequencing was used to assess the familial segregation of the variants. Also, the variants were sent for Sanger sequencing for validation if the in-house criteria for quality control were not met: the sequencing depth was below 20, the variant allele was present in less than 30% of the reads, or variant quality was flagged by the GATK VQSR algorithm.

The classification of variants was based on the criteria published by the American College of Medical Genetics (ACMG) (Richards et al., 2015).

The disease-causing variants reported in the databases ClinVar and HGMDPro were considered to be pathogenic.

The novel variants identified in our study were categorized as pathogenic or likely pathogenic if they met the following criteria: (1) *COL4A1/2* and *COL5A1* genes with missense variants replacing glycine with another amino acid in the triple helix domain, (2) genes with loss-of-function variants for which other reported loss-of-function variants have been reported as pathogenic (changes at the canonical splice site, nonsense variants, frameshift variants).

For example, in the ClinVar and HGMD pro databases loss-of-function variants for *COL4A1*, *COL4A2*, and *COL5A1* have been reported as pathogenic. As suggested by the ACMG criteria, sequence changes were classified as variants of unknown significance (VUS) if the characterictics were not met. In the 7S or NC1 domain in *COL4A1/2* or in the N-terminal or C-terminal region of *COL5A1* gene pathogenic variants were not found.

Genetic testing was performed at the Medical Laboratory of Tartu University Hospital, accredited under ISO 15189:2012.

6.2.3. Radiological evaluation

All US, CT and MRI scans were stored in the DICOM format in the population based Estonian Medical Picture Archive (www.pacs.ee) (Aavik et al., 2007) from 2004 and in the picture archive of Tartu Children's Hospital between 1994–2004 and were reviewed for the study.

The side, localization and first diagnosis of grade III IVH or PVHI or residual findings of PVHI on cerebral US were recorded.

The diagnosis of PVHI/PVI was confirmed by MRI examinations around at postmenstrual age of GW 40 or later during infancy. The follow-up MRI was performed without anesthesia in case MRI had not been done earlier for clinical purposes as part of a larger follow up study of patients with perinatal stroke at the age of 7–18 years. MRI data was acquired using a 3 Tesla Philips Achieva MR scanner with an 8-channel SENSE head coil (Philips Medical Systems, Best, The Netherlands). Fixed imaging research protocols were used in most cases.

On MRI, the side and localization of PVHI/PVI, the severity of enlargement of the lateral ventricles, the size of periventricular cyst and the residues of hemosiderin in the area of the germinal matrix, lateral ventricles and/or in periventricular lesion were evaluated (Takanashi et al., 2003; Bassan et al., 2006b; Kirton et al., 2008; Ilves et al., 2016; Fluss et al., 2019; eurUS.brain group et al., 2020). MRI findings, including the size of the basal ganglia and thalamus (normal/mild or moderate/severe decrease in size) and enlargement of the lateral ventricles (normal/mild or moderate/severe enlargement) were evaluated.

PVI/ PVHI lesions on MRI with involvement of several lobes were considered to be large lesions (Laugesaar et al., 2018; Lõo et al., 2018).

If CT images were available in the Estonian Picture Archive, the presence of calcifications was evaluated.

6.2.4. Clinical assessment of global outcome (Substudy III)

The study is part of a larger outcome study of perinatal stroke patients (Laugesaar et al., 2018; Lõo et al., 2018; Ilves et al., 2022b). During follow-up at the age of 7–18 years the patients were evaluated by child neurologists, clinical psychologists and a physiotherapist. MRI and genetic testing were included in the same follow-up in cases when follow-up MRI or genetic testing had not been performed for clinical purposes.

Neurodevelopmental outcome in children with perinatal stroke was evaluated according to the Pediatric Stroke Outcome Measure (PSOM) (Kitchen et al., 2012), a stroke-specific outcome measure, which scores right and left sensorimotor, language, cognitive and behavioral performances as measured by the child neurologist (R.L). A deficit score in PSOM ranges 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. The severity of motor deficit was evaluated as mild – no impact on function; moderate – some functional limitations; severe – missing function. Moderate to severe unilateral sensorimotor deficit corresponded to the definition of spastic hemiparesis (e.g. unilateral spastic cerebral palsy): unilateral increased muscle tone and pathological reflexes, resulting in an abnormal pattern of movement and posture (Cans, 2000).

Epilepsy was diagnosed by the treating child neurologist according to the following definition: at least 2 unprovoked seizures occurring >24 h apart or one unprovoked seizure with high recurrence risk, or diagnosis of the epilepsy syndrome (Fisher et al., 2014; Laugesaar et al., 2018). Due to the high recurrence risk of seizures in cases of pre-existing brain lesions as in perinatal stroke, epilepsy was often diagnosed after the first epileptic seizure if EEG was supportive.

6.3. Statistical analysis

Statistical evaluation was performed using the statistical package SAS version 9.4 (SAS Institute, Cary, NC) and the R Statistical Software (version 4.0.2).

The normality of data was evaluated using the Shapiro-Wilk test (Substudies I–III).

Continuous values are summarized as medians with the interquartile range (IQR) and range (minimum, maximum), and categorical data, as absolute counts and percentages (Substudies I–III) and means with the 95% confidence interval (CI) (Substudies I–III). Qualitative variables are presented as absolute and relative frequencies (Substudies I–III).

Differences between the groups were analyzed by the nonparametric Mann-Whitney *U* test (Substudies I, III) and by the nonparametric Kruskal-Wallis test (Substudy II) for continuous variables.

Univariate comparison was analysed using the χ^2 test or Fisher's exact test, multinomial logistic regression for dichotomous variables, as appropriate (Substudies I, II, III). The odds ratio (OR) with the 95% CI was estimated as the measure of association (Substudies I-III). All raw *P* values are two-sided. Alpha level for determining significance is $p = 0.05$ (substudies II and III).

Correction for multiple comparisons was used to control false discovery rate (FDR) (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.

For a set of risk factors, the cut-off *P* value for significance of a single comparison is 0.012 in Substudy I. A multiple logistic regression model was developed to determine independent predictors of presumed antenatal PVI in Substudy I. The results of the logistic regression model is shown as the adjusted odds ratio (aOR) with the 95% CI.

In Substudy II univariate comparison was analyzed for dichotomous variables, using multinomial logistic regression. All findings were adjusted for the year of birth and gestational age at birth and, except for findings with low frequency like *in vitro* fertilization, no intubation at birth or hypotermia on admission. Multiple testing was corrected using the FDR linear step-up procedure (Benjamini and Hochberg, 1995). The cut-off p value for significance of a single comparison for a set of risk factors was 0.002 between the antenatal onset versus unknown time-onset group, 0.026 between the antenatal versus neonatal onset group, 0.009 between the unknown time-onset versus neonatal onset group, 0.021 between the antenatal onset group versus controls, 0.004 between the neonatal onset group versus controls, 0.012 between the unknown time- onset group versus controls.

A multiple logistic regression model was developed in Substudy II to describe the influence of different risk factors on IVH/PVHI for the different study groups and controls. Variables with $p<0.2$ in univariate comparison were entered in a stepwise multiple logistic regression model, the output of the model was presented as the adjusted odds ratio (aOR) with the 95% CI.

In Substudy III, for significance of a single comparison for a set of pregnancy and delivery risk factors, the threshold was set at 0.0028 and for a set of MRI findings it was set at 0.035.

6.4. Ethical approval and declarations

The studies were approved by the Ethics Review Committee on Human Research of the University of Tartu.

The Happy Pregnancy Study for the control group was approved by protocol no 221/T-6, 17.12.2012 and no 286/M-18, 15.10.2018 and all recruited participants signed a form of written informed consent. The study of perinatal stroke was approved by protocols no 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). The data of the risk factors for children with PVHI or presumed PVI were collected retrospectively and the committee waived the need for patient consent.

All procedures performed within the studies 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 provided by all individual participants older than seven years and by their parents during follow-up investigations including genetic testing and MRI examination.

7. RESULTS

7.1. Pregnancy and delivery risk factors for PVHI/PVI (Substudy I)

7.1.1. Demographics (Substudy I)

The final study group consisted of 43 mothers and term or near-term (GW ≥36) born children with presumed antenatal PVI, and 86 mothers and preterm (GW <36) children with PVHI; the control group consisted of 2,168 pregnant women and their unaffected term or near-term (GW ≥36) neonates. The representative demographic data of the groups is shown in Table 1.

The study groups were similar in terms of maternal age, number of pregnancies, deliveries and mode of conception (Table 1). The children of the presumed antenatal PVI and control groups were well comparable regarding attended prenatal care, Apgar scores, gestational age, weight, and head circumference at birth. In the group of presumed antenatal PVI, all pregnancies were monitored. On the other hand, 8/86 (9%) pregnancies were not monitored before preterm birth in the group of PVHI (odds ratio (OR), 9 [95% confidence interval (CI), 0.5–167], p=0.05). In the group of PVHI, 37/86 (43%) neonates died during the first month.

Table 1. Maternal, pregnancy and delivery related data

	Controls N=2168	Presumed antenatal PVI N=43	Presumed antenatal PVI versus Controls (p)	PVHI N=86	Presumed antenatal PVI versus PVHI (p)
Maternal age, y, [IQR]	29.3 [26–33]	28 [23–34]	0.12	28 [23–32]	0.83
Gravidity, n, [IQR]	2 [1–3]	2 [1–3]	0.24	3 [1–5]	0.04
Parity, n, [IQR]	2 [1–2]	2 [1–2]	0.49	2 [1–3]	0.70
Threatened pregnancy loss, n (%); GW, [IQR]	197 (9.1) 10.6 [8–14]	3 (7.0) 17 [16–20]	0.89 0.02	17 (20) 13 [9–17]	0.07 0.14
Imminent preterm delivery, n (%); GW, [IQR]	77 (3.6) 30.9 [27–33]	5 (12) 34 [25–34]	0.02 0.94	15 (17) 24 [22–27]	0.39 0.02
Apgar score at 5 min, [IQR]	9 [9–9]	9 [9–9]	0.02	6 [4–7]	<0.001*
Range	1–10	7–10		0–1	
Boys, n (%)	1138 (53)	20 (47)	0.40	56 (65)	0.05
Gestational age at birth, GW, [IQR]	40 [39–40]	40 [38–40]	0.30	27 [25–30]	<0.001*

	Controls N=2168	Presumed antenatal PVI N=43	Presumed antenatal PVI versus Controls (p)	PVHI N=86	Presumed antenatal PVI versus PVHI (p)
Birth weight, g, [IQR]	3578 [3290–3894]	3405 [3120–3745]	0.07	1060 [790–1500]	<0.001*
Head circumference, cm, [IQR]	35 [34–36]	35 [34–36]	0.56	26 [25–30]	<0.001*
Death within 28 days, n (%)	N/A	0 (0%)	N/A	37 (43%)	<0.001*

(Gravidity, total number of pregnancies including index pregnancy; parity, number of births including index birth; GW, gestational weeks; N, number of subjects; n, number of observations; IQR, interquartile range; OR, odds ratio; CI, confidence interval for odds ratio; N/A, not applicable; g, grams; cm, centimeters, y, years).
*statistically significant difference for a single comparison was set at 0.012.

7.1.1.1. Risk factors in the groups of presumed antenatal PVI and control

The mothers of the group of presumed anetantal PVI showed significantly higher prevalence of maternal bacterial infections during pregnancy compared to control ($p<0.001$; Figure 6, Table 2). More specifically, compared to the control group, they were more often diagnosed with GTI ($p=0.001$), including pyelonephritis ($p<0.001$). The difference in the incidence of maternal pyelonephritis between the group of presumed antenatal PVI (10/43; 23%) and the control group (15/2168; 0.7%) was greater than 40 fold (OR, 43 [95% CI, 16–112], $p<0.001$).

Although the mothers of children with presumed antenatal PVI had significantly more often ($p<0.05$) imminent preterm delivery at GW ≥22 and preeclampsia, and delivered more often small for gestational age neonates compared to control mothers (Figure 4), these findings were not significantly different after FDR control. Both preeclampsia and infection were found in ten mothers (10/2168; 0.5%) of the control group and in only one mother (1/43; 2.3%) of the group of presumed antenatal PVI, the difference being not statistically different ($p=0.19$). There was no difference between controls and children with presumed antenatal PVI in the incidence of delivery related acute stress risk factors, such as mode of delivery, fetal dystocia, placental abruption and chorioamnionitis (Figure 6).

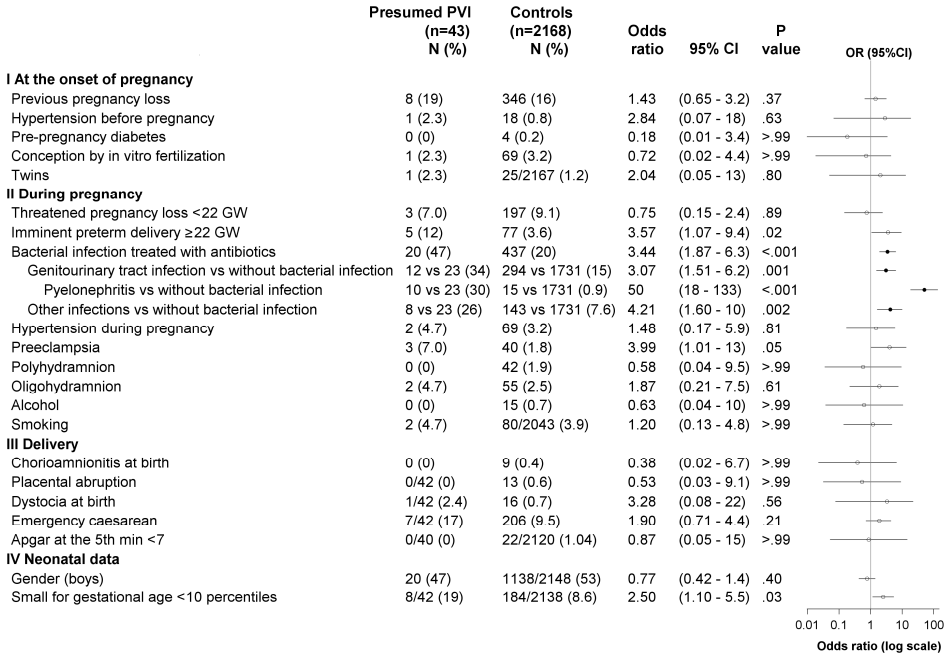

Figure 6. Comparison of the risk factors between the groups of presumed antenatal PVI and control. False discovery rate analysis revealed that the cut-off p value for significance of a single comparison for a set of risk factors was 0.012. (CI, confidence interval; GW, gestational weeks; PVI, periventricular venous infarction; N, number of patients). (Ilves et al., 2022, published with permission from Sage Journals).

7.1.1.2. Risk factors in the groups of presumed antenatal PVI and PVHI

Maternal bacterial infection was frequent in the groups of both presumed antenatal PVI (20/43; 47%) and PVHI (42/86; 49%) (p =0.72) (Figure 7, Table 2).

The prevalence of GTI, including maternal pyelonephritis, was similar in the groups of presumed PVI (10/43; 23%) and PVHI (15/86; 17%) (OR, 1.4 [95% CI, 0.6–3.5], p=0.43).

The group of presumed antenatal PVI showed a significantly lower incidence of twin births, male newborns, and birth distress-related factors, such as chorioamnionitis, placental abruption, emergency caesarean section, and 5-minute Apgar scores <7 (Figure 7). However, FDR control showed that the difference in the proportion of male newborns was not significant.

	Presumed PVI (n=43) N (%)	Neonatal PVHI (n=86) N (%)	Odds ratio	95% CI	P value
I At the onset of pregnancy					
Previous pregnancy loss	8 (19)	34 (40)	0.32	(0.13 - 0.79)	.01
Hypertension before pregnancy	1 (2.3)	7 (8.1)	0.27	(0.01 - 2.2)	.37
Pre-pregnancy diabetes	0 (0)	2 (2.3)	0.39	(0.02 - 8.3)	.55
Conception by in vitro fertilization	1 (2.3)	5 (5.8)	0.39	(0.01 - 3.6)	.69
Twins	1 (2.3)	20 (23)	0.08	(0.002 - 0.54)	.002
II During pregnancy					
Threatened pregnancy loss <22 GW	3 (7.0)	17 (20)	0.30	(0.08 - 1.10)	.07
Imminent preterm delivery ≥22 GW	5 (12)	15 (17)	0.62	(0.21 - 1.9)	.39
Bacterial infection treated with antibiotics	20 (47)	42 (49)	0.91	(0.44 - 1.9)	.80
Genitourinary tract infection vs without bacterial infection	12 vs 23 (34)	32 vs 44 (42)	0.72	(0.31 - 1.7)	.43
Pyelonephritis vs without bacterial infection	10 vs 23 (30)	15 vs 44 (25)	1.28	(0.50 - 3.3)	.62
Other infections vs without bacterial infection	8 vs 23 (26)	10 vs 44 (19)	1.53	(0.53 - 4.4)	.43
Hypertension during pregnancy	2 (4.7)	0 (0)	10	(0.49 - 222)	.11
Preeclampsia	3 (7.0)	7 (8.1)	0.85	(0.13 - 4.0)	>.99
Polyhydramnion	0 (0)	4 (4.7)	0.21	(0.01 - 4.0)	.30
Oligohydramnion	2 (4.7)	9 (10.5)	0.42	(0.04 - 2.2)	.45
Alcohol	0 (0)	3 (3.5)	0.27	(0.01 - 5.4)	.55
Smoking	2 (4.7)	10 (12)	0.37	(0.04 - 1.9)	.34
III Delivery					
Chorioamnionitis at birth	0 (0)	46/84 (55)	0.01	(0.001-0.16)	<.001
Placental abruption	0/42 (0)	28/85 (33)	0.02	(0.001 - 0.40)	<.001
Dystocia at birth	1/42 (2.4)	44 (51)	0.19	(0.004 - 1.4)	.14
Emergency caesarean	7/42 (17)	37 (43)	0.27	(0.11 - 0.66)	.005
Apgar at the 5th min <7	0/40 (0)	52 (63)	0.01	(0.0004 - 0.13)	<.001
IV Neonatal data					
Gender (boys)	20 (47)	56 (65)	0.47	(0.22 - 0.98)	.04
Small for gestational age <10 percentiles	8/42 (19)	15 (17)	1.11	(0.43 - 2.9)	.82

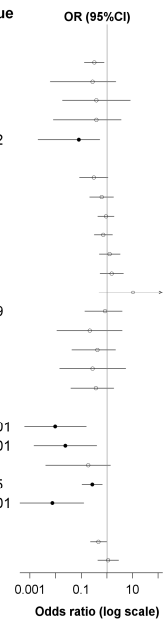

Figure 7. Comparison of pregnancy and delivery risk factors between the groups of presumed antenatal PVI and neonatal PVHI. False discovery rate analysis showed that the cut-off p value for significance of a single comparison for a set of risk factors was 0.012. (CI, confidence interval; GW, gestational weeks; PVHI, periventricular hemorrhagic infarction; PVI, periventricular venous infarction; N, number of patients). (Ilves et al., 2022, published with permission from Sage Journals).

7.1.1.3. Multiple logistic regression analysis in Substudy I

Based on multiple logistic regression analysis, GTI (aOR, 3.1 [95% CI, 1.5–6.2], p=0.0015), other bacterial infections, except for GTI (aOR, 4.4 [95% CI, 2.0–9.8], p=0.0003) and preeclampsia (aOR, 4.3 [95% CI, 1.3–14], p=0.015), were independent predictors of presumed antenatal PVI compared to control.
Multiple logistic regression analysis revealed that twin pregnancy (aOR, 8.3 [95% CI, 1.4–48], p=0.018) and previous spontaneous pregnancy loss (aOR, 3.0 [95% CI, 1.2–7.6], p=0.02) were independent predictors of PVHI versus presumed antenatal PVI.

7.1.2. Bacterial infection during pregnancy (Substudy I)

GTI, including asymptomatic bacteriuria, accounted for at least 60% of all infections during pregnancy in the mothers of all study groups in Substudy I (control, presumed antenatal PVI in term born children and PVHI in preterm children) (Table 2). The proportions of GTI and infections of other organ systems did not differ between the three study groups.

Table 2. Detailed data on bacterial infections in the study groups.

	Controls N=437/2168	Presumed PVI N=20/43	Presumed PVI versus Controls (p)	PVHI N=42/86	Presumed PVI versus PVHI (p)
Diseases of respiratory system n (%)	100 (22.9)	6 (30)	0.43	6 (14.3)	0.18
Pneumonia n (%)	1 (0.2)	0 (0)	>0.99	1 (2)	>0.99
Diseases of digestive system n (%)	26 (6)	1 (5)	>0.99	1 (2)	0.54
Diseases of oral cavity and salivary gland n (%)	23 (5.3)	0 (0)	0.61	1 (2.4)	>0.99
Appendicitis n (%)	3 (0.7)	1 (5)	0.16	0 (0)	0.32
Diseases of genitourinary tract n (%)	294 (67.3)	12 (60)	0.50	32 (76.2)	0.19
Pyelonephritis n (%)	15 (3.4)	10 (50)	<0.001*	15 (36)	0.28
Asymptomatic bacteriuria n (%)	166 (38)	0 (0)	<0.001*	0 (0)	N/A
Chlamydia n (%)	34 (8)	0 (0)	0.39	3 (7)	0.54
Other genitourinary infections n (%)	79 (18)	2 (10)	0.55	6 (14)	>0.99
Chorioamnionitis n (%)	0 (0)	0 (0)	N/A	8 (19)	0.05
Other bacterial infections (ear, skin, varicose veins, etc. n (%)	17 (3.9)	1 (5)	0.56	3 (7.1)	>0.99
Time of bacterial infection GW, [IQR]	16.4 [12.4–26.1]	26.5 [21–31]	0.001*	23.5 [21.5–27]	0.24
Bacterial infection GW <32, n (%)	376/437 (86%)	15/18 (83%)	0.73	38/40 (95%)	0.17
Bacterial infection GW <21, n (%)	289/437 (66%)	3/18 (17%)	<0.001*	9/40 (23%)	0.74

N is the number of women with bacterial infections treated with antibiotics during pregnancy/all women. Data about infection is presented as n (%) of all infections in the group. GW, gestational weeks; IQR, interquartile range.

*$p<0.014$ was significant after control for false discovery rate for presumed antenatal PVI versus control and $p<0.005$ was significant for presumed versus neonatal PVI.

The control group included 166/437 (38%) mothers with asymptomatic bacteriuria among the infections requiring antibiotic treatment. At the same time, neonates with PVHI and term born children with presumed antenatal PVI did not present such asymptomatic cases (OR, 25 [95% CI, 1.5–418], p<0.001).

In contrast, only 15/437 (3.4%) control mothers with infection suffered from pyelonephritis, compared to 10/20 (50%) mothers of the group of presumed antenatal PVI (OR, 28 [95% CI 10–78], p<0.001).

The proportion of pyelonephritis in the group of presumed antenatal PVI and in the group of PVHI was similar, 10/20 (50%) versus 15/42 (36%), respectively (OR, 1.8 [95% CI, 0.6–5.3], p=0.28).

7.1.3. Time of infection (Substudy I)

There was no difference between the groups of presumed antenatal PVI and PVHI in the median (IQR) gestational age of the first maternal bacterial infection (OR, 26.5 [95% CI, 21–31] versus OR, 23.5 [95% CI, 21.5–27] GW, respectively; p=0.24). However, the infections were diagnosed earlier in pregnancy in the control group (OR, 16.4 [95% CI, 12.4–26.1], p=0.001) compared to the group of presumed antenatal PVI. Furthermore, bacterial infections before GW 21 were significantly more prevalent among controls (249/428; 58%) compared to cases with presumed antenatal PVI (3/18; 17%) (OR, 7 [95% CI, 2–24], p<0.001).

Data on the first bacterial infection, imminent preterm delivery and birth in relation to gestational age is presented in Figure 8.

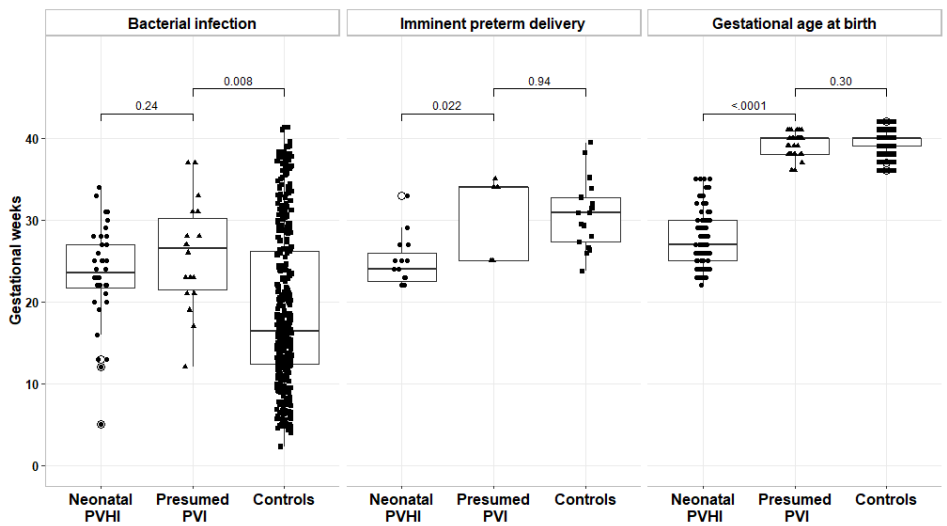

Figure 8. Individual data about gestational age at the time of bacterial infection, imminent preterm delivery and birth. (PVHI, periventricular hemorrhagic infarction; PVI periventricular venous infarction). (Ilves et al., 2022, published with permission from Sage Journals).

7.2. Pregnancy and delivery risk factors for antenatal development of PVHI (Substudy II)

7.2.1. Demographics (Substudy II)

In the final study group, 27/120 (22.5%) cases of severe IVH/PVHI were considered antenatal in origin, including 20 neonates in whom IVH/PVHI was visible already on the first cerebral US performed within 12 h of age and 7 neonates with cystic changes visible in the area of PVHI within 3 days of life. In 70/120 (58%) neonates US investigations suggested that severe IVH/PVHI developed during the neonatal period. In 23/120 (19%) cases the time of the first US failed to make the emergence of severe IVH/PVHI conclusive for judging if its onset was antenatal or neonatal, and these cases formed the unknown time-onset group. The demographic data for the three study groups and as well as for the control group (n=50) is shown in Table 3.

The mothers of the neonates in the groups of antenatal onset, unknown time-onset and neonatal onset, and in the control group were similar regarding several characteristics: age, number of previous deliveries, threatened pregnancy losses; and the neonates were similar regarding sex distribution; birth weight, head circumference and Apgar score, as well as SNAPPE score within the first 24 hours.

However, compared to the groups of children with antenatal, unknown time-onset and neonatal onset of IVH/PVHI, the neonates of the control group had significantly higher gestational age, larger birth weight and head circumference and their mothers had a higher number of imminent preterm deliveries at GW ≥22.

Table 3. Maternal, pregnancy and delivery related risk factors in preterm born children with antenatal or neonatal onset of severe IVH/PVHI (The data in the table is presented as median (interquartile range) if not stated otherwise).

	Controls N=50	Antenatal onset N=27	Unknown time-onset N=23	Neonatal onset N=70	Overall P value
Maternal age, y	30 [26–32]	27 [24–31]	28 [23–31]	26 [22–32]	0.12
Gravidity, n,	2 [1–2]	3 [1–4]	3 [1–6]	2.5 [1–5]	0.011[bc]
Parity, n,	1 [1–2]	1 [1–3]	2 [1–3]	2 [1–3]	0.12
Threatened pregnancy loss, n (%);	14 (28)	5 (19)	6 (26)	12/69 (17)	0.51
gestational weeks	8.2 [6.9–10.5]	12 [8–17]	15.5 [9–19]	13 [11–20]	0.17
Imminent preterm delivery, n (%);	23 (46)	6 (22)	5 (22)	8/69 (12)	0.0003[c]
gestational weeks	30.7 [30.6–31.6]	25.5 [23–27]	25.0 [25–25]	22.5 [22–24.5]	0.008[bc]
Apgar score at 5 min,	8 [7–9]	6 [4–7]	6 [5–7]	6 [4–7]	<.001[abc]
Boys, n (%)	29 (58)	17 (63)	17 (74)	42 (60)	0.60
Gestational age at birth, weeks	33 [30–34]	28 [24–30]	27 [24–30]	26 [25–29]	<.001[abc]
Birth weight, g	1945 [1390–2246]	1100 [760–1526]	1040 [750–1520]	930 [744–1310]	<.001[abc]
Birth weight <1500g, n (%)	16 (32)	20 (74)	16 (70)	60 (86)	<.001[abc]
Head circumference, cm,	31 [29–32.5]	28 [25.5–29.3]	26.5 [25–30]	25 [24–27.5]	<.001[abc]
SNAPPE II	N/A	60 [30–94]	29 [28–75]	62 [43–76]	0.22

(Gravidity, total number of pregnancies including index pregnancy; parity, number of births including index birth; GW, gestational weeks; N, number of subjects; n, number of observations; OR, odds ratio; CI, confidence interval for the odds ratio; N/A, not applicable; g, grams; cm, centimeters; y, years; SNAPPE II, score for neonatal acute physiology and SNAP perinatal extension).

Comparisons are made using the Kruskal Wallis test for continuous variables and multinomial logistic regression for dichotomous variables. Statistically significant difference for a single comparison is set at 0.029 (false discovery rate).

[a]Antenatal onset versus control

[b]Unknown time-onset versus control

[c]Neonatal onset versus control

7.2.2. Risk factors and complications in preterm born children of the groups of IVH/PVHI

The group of antenatal onset of IVH/PVHI showed significantly higher prevalence of maternal bacterial infections than the group of neonatal onset (aOR, 5.7 [95% CI, (2.1–16], p=0.0008) (Figure 9).

GTI, including pyelonephritis, were more often diagnosed in the mothers of the group of antenatal onset, compared to the group of neonatal onset (p=0.005), and this was also the case with the other infections (p=0.006). However, after FDR control pyelonephritis alone was not significantly different between the groups of antenatal and neonatal onset.

The group of antenatal onset had also more often clinical signs of chorioamnionitis (p=0.025) and histological chorioamnionitis (p=0.025), compared to the group of neonatal onset.

However, regarding the other pregnancy, delivery related and neonatal risk factors, no differences were found between the groups.

The neonates of the group of antenatal onset and the group of unknown time-onset, compared to the group of neonatal onset, showed significantly less risk factors related to fetal or neonatal stress and complications, inclucing emergency caesarean section, need for cardiotonic/vasoactive treatment and artificial ventilation more than >24h.

The need for surfactant treatment as well as the need for medical or surgical treatment for persistent arterial duct, but also neonatal seizures were more frequent in the group of neonatal onset (Figure 9).

No need for intubation during primary hospitalization and pneumothorax was recorded more frequently in the group of antenatal onset, compared to the group of neonatal onset (Figure 9).

Interestingly, some of the children in the groups of antenatal onset and unknown time-onset were never intubated: 4/27 (15%) and 4/23 (17%), respectively, which is significantly more compared to the group of neonatal onset (p=0.006 and p=0.004, respectively) (Figure 9).

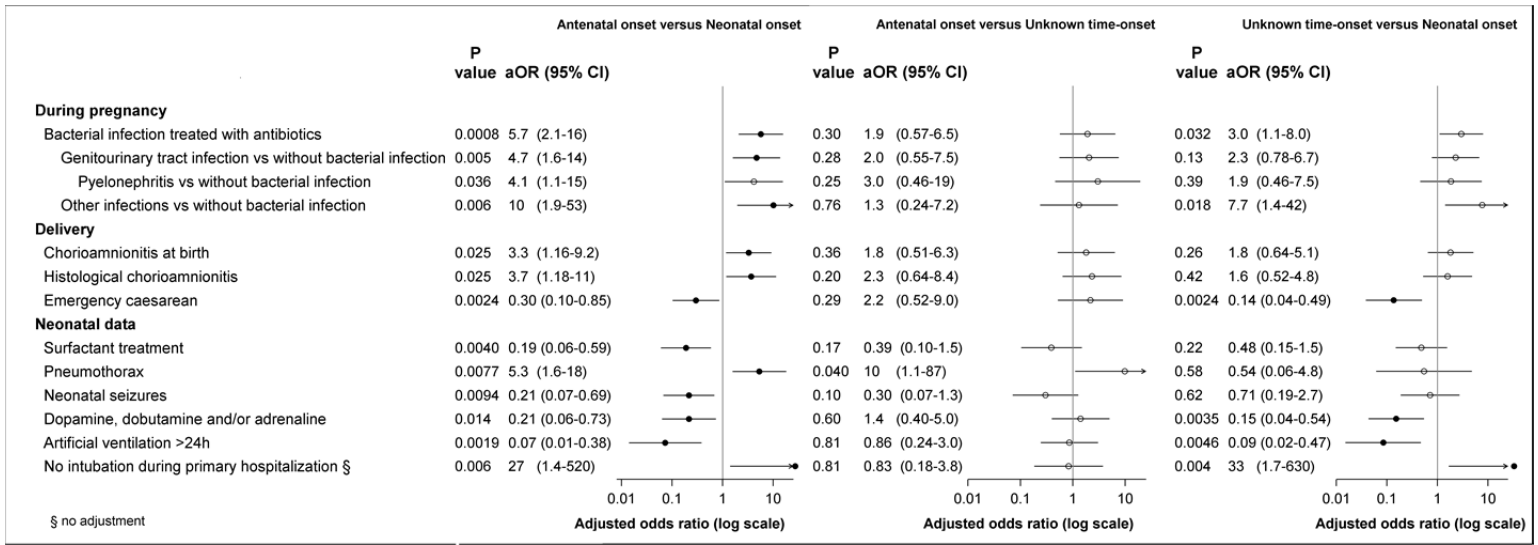

Figure 9. Comparison of the pregnancy, delivery and neonatal risk factors in the groups of antenatal, unknown time-onset and neonatal onset IVH/PVHI. (aOR, adjusted odds ratio; CI, confidence interval for the odds ratio; h, hours). Only statistically significant characteristics are presented. All findings except for the low frequency finding (no intubation) were adjusted for gestational age and year of birth. The cut-off p value for significance of a single comparison for a set of risk factors' false discovery rate is 0.026 between the groups of antenatal onset and neonatal onset; 0.002 between the groups of antenatal onset and unknown time-onset; and 0.009 between the groups of unknown time-onset and neonatal onset. (Ilves et al., 2024, published with permission from IOS Press).

7.2.3. Risk factors and complications in the groups of IVH/PVHI and controls

Maternal bacterial infections, including GTI, were found significantly more often in the groups of antenatal onset and unknown time-onset compared to the control group ($p=0.005$ and $p=0.01$, respectively). Also, pyelonephritis and clinical signs of chorioamnionitis at birth were noted significantly more often in the mothers of the group of antenatal onset, compared to the mothers of the control group, however, after FDR correction pyleonephritis was not significant (Figure 10).

There were no differences between the study groups and the control group regarding the other prenatal and delivery rated risk factors.

The findings presented are adjusted for gestational age and the year of birth, except for gestational age, which is adjusted only for the year of birth. For a single comparison of risk factors, the cut-off P value for significance of a single comparison for a set of risk factors is 0.021 between the groups of antenatal onset and controls; 0.012 between the groups of unknown time-onset and controls; and 0.004 between the groups of neonatal onset and controls. The figure presents only statistically significant variables.

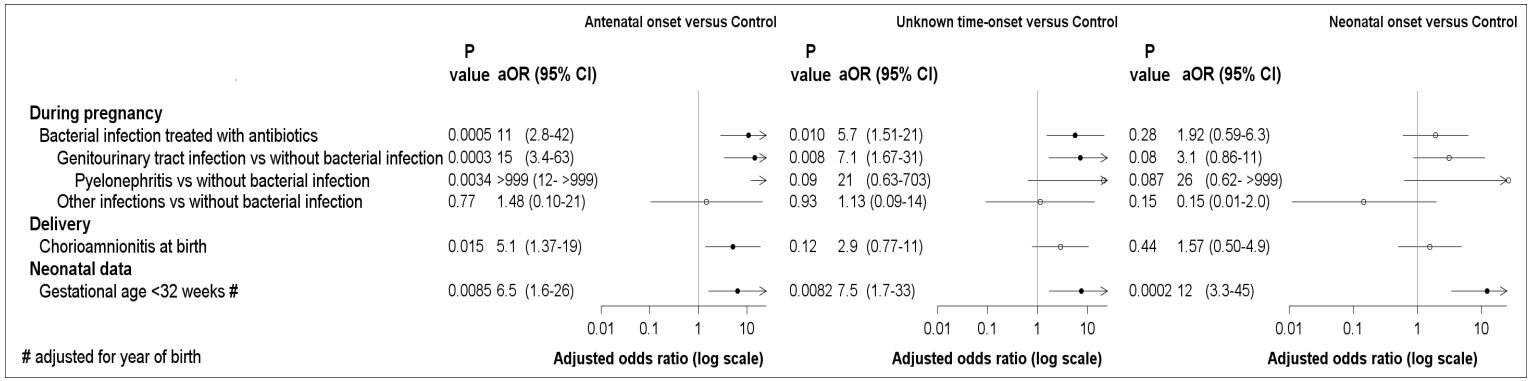

Figure 10. Pregnancy and delivery related risk factors in the groups with different IVH/PVHI onset compared to the control group. (aOR, adjusted odds ratio; CI, confidence interval for the odds ratio). (Ilves et al., 2024, published with permission from IOS Press).

7.2.4. Bacterial infection during pregnancy in preterm born children with PVHI (Substudy II)

GTI was the most common bacterial infection in the mothers of the study groups (antenatal onset, unknown time-onset and neonatal onset) and the control group, accounting for 63.6% of all infections in all groups during pregnancy. The proportions of GTI and the other infections across all study groups were similar (Table 4).

Infection occurred significantly earlier in the mothers of the control group compared to the mothers of the study groups ($p<0.001$). In the control group 81.8% of the mothers had bacterial infection before GW 21 compared to only 15.8% of the mothers in the group of antenatal onset, 35.7% of the mothers in the group of unknown time-onset and 13% of the mothers in the groups of neonatal onset ($p=0.019$) (Table 4).

Based on multiple logistic regression analysis adjusted for gestational age and the year of birth, maternal bacterial infection (aOR, 5.8 [95% CI, 2.0–17], $p=0.0013$) and no need for surfactant treatment (aOR, 6.1 [95% CI, 1.7–22] $p=0.0054$; were the predictors in the group of antenatal onset IVH/PVHI compared to the group of neonatal onset.

Based on multiple logistic regression analysis adjusted for gestational age and the year of birth, maternal bacterial infection (aOR ,14 [95% CI, 2.1–98], $p=0.0067$), chorioamnionitis at birth (aOR, 15 [95% CI, 1.5–143], $p=0.021$), and no signs of imminent preterm delivery (aOR, 8.2 [95% CI, 1.1–61] $p=0.039$) were the independent predictors of antenatal onset IVH/PVHI compared to control.

Compared to control, the only remaining independent predictor in the group of neonatal onset IVH/PVHI was emergency caesarean section (aOR, 6.5 [95% CI,1.2–36], $p=0.003$).

Table 4. Data on bacterial infections in the study groups adjusted for the year of birth and gestational age.

	Controls	Antenatal onset	Unknown time-onset	Neonatal onset	P value
Number of patients with infections/Number of patients (%)	N=11/50 (22%)	N= 20/27 (74%)	N=14/23 (61%)	N= 23/69 (33%)	
Infections of respiratory system, n (%)	1 (9.1)	2 (10)	3 (21.4)	2 (8.7)	0.56
Infections of digestive system including oral cavity, n (%)	1 (9.1)	1 (5)	0 (0)	0 (0)	0.31*
Infections of genitourinary tract, n (%)	7 (63.6)	15 (75)	10 (71)	20 (87)	0.10

Number of patients with infections/Number of patients (%)	Controls N=11/50 (22%)	Antenatal onset N= 20/27 (74%)	Unknown time-onset N=14/23 (61%)	Neonatal onset N= 23/69 (33%)	P value
Pyelonephritis, n (%)	0 (0)	7 (35)	4 (28.6)	10 (43.5)	0.55
Asymptomatic bacteriuria, n (%)	2 (18)	0 (0)	0 (0)	0 (0)	>.99
Chlamydia , n (%)	3 (27.3)	0 (0)	1 (7.1)	3 (13)	0.81
Other genitourinary infections, n (%)	2 (18)	5 (25)	2 (14.3)	3 (13)	0.71
Chorioamnionitis, n (%)	0 (0)	3 (15)	3 (21.4)	4 (17.4)	0.98
Other bacterial infections (ear, skin, varicose veins, etc.), n (%)	2 (18)	2 (10)	1 (7.1)	1 (4.3)	0.74
Time of bacterial infection GW, median [IQR]	8.1 [6.0–19.4]	26.0 [21–28]	23.0 [20–25]	23.0 [22–25]	<0.001 [ab]
Bacterial infection GW <21, n (%)	9 (81.8)	3/19 (15.8)	5 (35.7)	3 (13.0)	0.019 [ab]

GW-gestational week, IQR-intequartile range,
N represents the number of bacterial infections treated with antibiotics during pregnancy/all patients. Data about infection are presented as n (%) of all infections in the group. *No adjustment.
Statistically significant difference for a single comparison was set at 0.009 (false discovery rate). [a]Antenatal onset versus control; [b]Neonatal onset versus control

7.3. Genetic risk factors for perinatal stroke (Substudy III)

7.3.1. Demographics of the groups of genetic risk factors (Substudy III)

The final cohort consisted of 85 PVHI/PVI patients and their representative demographic data is shown in Table 6. A total of 39 preterm born children with PVHI, six children with antenatal PVI, and 40 children with presumed antenatal PVI were included in the study. In terms of maternal age at delivery, previous pregnancy loss, number of pregnancies, deliveries, mode of conception, child gender, number of small-for-gestational-age patients, and number of patients suffering from moderate/severe motor deficits or epilepsy, the study groups were comparable. However, the group of presumed antenatal PVI showed significantly lower incidence of emergency caesarean section and 5-minute Apgar score <7 compared to the group of PVHI (Table 5).

Table 5. Demographics and distribution of genetic variants associated with stroke in different PVHI/PVI subgroups in Substudy III

	Total N=85	Preterm PVHI N=39	Antenatal PVI N=6	Presumed PVI N=40	P value
Gestational age at birth, GW, [IQR]		28.0 [26–32]	39.0 [38–39]	40.0 [38.5–40]	<.001*
Maternal age at delivery, y, [IQR]		27.0 [24–32]	27.5 [26–31]	28.0 [23–35]	0.91
Previous pregnancy loss, n (%)		15/37 (41)	4 (67)	7 (18)	0.022
Gravidity, n, [IQR]		3 [1–5]	3.5 [2–4]	2 [1–3]	0.18
Parity, n, [IQR]		2 [1–3]	1.5 [1–3]	2 [1–2]	0.98
Conception by in vitro fertilization, n (%)		3 (7)	0 (0)	1 (3)	0.52
Emergency caesarean, n (%)		19 (49)	0 (0)	6 (15)	0.0013*#
Apgar at the 5th min <7, n (%)		18 (46)	0 (0)	0 (0)	<.001*
Gender (boys), n (%)		24 (62)	3 (50)	19 (48)	0.46
Small for gestational age <10 percentiles, n (%)		8 (21)	2 (33)	7/39 (18)	0.76
Head circumference at delivery <10 percentiles, n (%)		3/27 (11)	3 (50)	2/30 (7)	0.026
Death <28 days, n (%)		2 (5)	0 (0)	0 (0)	0.34
Moderate/severe motor deficit, n (%)		7/13 (54)	4 (67)	9/39 (23)	0.051
Epilepsy, n (%)		11/36 (31)	3 (50)	3 (8)	0.021
Pathogenic/likely pathogenic variants					
Collagenopathy, n (%)	7 (8.2)	1 (2.6)	2 (33.3)	4 (10.0)	0.043
Other stroke genes, n (%)	2 (2.4)	1 (2.6)	0 (0)	1 (2.5)	>0.99
Coagulopathy, n (%)	2 (2.4)	1 (2.6)	0 (0)	1 (2.5)	>099
Variants of unknown significance					
Collagenopathy, n (%)	7 (8.2)	4 (10.3)	0 (0)	3 (7.5)	0.68
Other variants, n (%)	5 (5.8)	3 (7.7)	0 (0)	2 (5)	0.78
Total reported variants	23 (27.1)	10 (25.6)	2 (33.3)	11 (27.5)	

Gravidity, total number of pregnancies including index pregnancy; parity, number of births including index birth; GW, gestational weeks; IQRm interquartile range; N, number of subjects; n, number of observations; N/A, not applicable; PVHI, periventricular hemorrhagic infarction; PVI, periventricular venous infarction; g, grams; cm, centimeters; y, years. *p<0.008 is significant after FDR control for presumed antenatal PVI versus preterm PVHI and #p<0.008 is significant for presumed antenatal PVI versus antenatal PVI.

Pathogenic and likely pathogenic variants in the genes associated with collagenopathy and coagulopathy were identified in 11/85 (12.9%) patients with PVHI/PVI (Table 5), of them *COL4A1/A2* and *COL5A1* variants in 7/11 (63%) children. In two patients with antenatal PVI and in three with presumed antenatal PVI a pathogenic collagen variant in *COL4A1* gene was detected; in one patient with presumed antenatal PVI, *COL4A2* gene was changed and in

one preterm born patient with PVHI *COL5A1* gene variant was found (Table 5). Genetic causes of stroke were not diagnosed until this study in three of these children, as these cases had been considered too mild to suspected any genetic disease.

In most of the severe cases the genetic cause had already been clinically suspected and genetic testing had been performed. However, almost half of the cases (5 of 11) with pathogenic or likely pathogenic variants had not been tested earlier, and among them three showed collagenopathy.

All sequence changes detected in this group were unique and variants of *COL4A1* gene were found most often (5 patients). Six of the 11 detected variants were described for the first time in this study.

We found only 2/85 (2.4%) children with the pathogenic variants connected with coagulopathy. One child with presumed antenatal PVI had the common prothrombin 20210G>A (rs1799963) risk variant and one preterm born child with PVHI and antithrombin 3 deficiency had the *SERPINC1* variant inherited from the father who was also known to be affected by thrombophilia (Table 5).

Further, there were 2/85 (2.4%) patients with variants in other genes which have earlier been associated with stroke: pathogenic findings in *NOTCH3* gene inherited from father to son with presumed antenatal PVI and the *MT-TL1* variant in one preterm born patient with PVHI.

In addition, variants of unknown significance were found in 12/85 (14.1%) patients with PVHI/PVI, both in collagen genes (*COL4A2, COL5A1, COL1A1*) and in other genes *(ATP1A, PHACTR1, FV, ITGA2B)*.

Seven patients had variants of unknown significance in collagen genes. A change in *COL5A1* was found in two preterm born patients with PVHI and in one child with presumed antenatal PVI; variant of *COL4A2* gene was found in a child with presumed antenatal PVI and the C*OL1A1* variant was found in two preterm children with PVHI and in one patient with presumed antenatal PVI.

Only two patients with coagulopathy associated genes of unknown significance, one of them with presumed antenatal PVI, had the *ITGA2B* variant and one preterm born patient with PVHI had a single *Factor V* gene variant. Additionally, 3/85 (3.5%) patients had variants of unknown significance in other genes which may be associated with stroke: two patients had the *ATP1A2* variant, and one had the *PHACTR1* variant.

7.3.2. Prenatal risk factors other than genetic in the group of collagenopathies and in the group without pathogenic gene variants

Children with collagenopaties (*COL4A1/A2* and *COL5A1*) (n=7) and children without any genetic changes (n=62), were well comparable in terms of maternal age, number of pregnancies, deliveries, mode of conception and risk factors during pregnancy and delivery (Figure 11). The mode of delivery, Apgar scores and need for resuscitation at birth were also similar between the groups; no

children with collagenopathy needed intubation or long term ventilation after birth. Remarkably, only one of the seven children with collagenopathies had seizures during the neonatal period, which was not different from the comparison group of children without genetic changes.

Furthermore, there were no differences between the groups in the number of children who were small for gestational age (<10 percentiles) at birth according to national normal values (Sildver et al., 2015). Although more children 3/5 (60%) with collagenopathy had a head circumference of <10 percentiles at birth compared to those without detected gene variants 5/46 (11%) (p=0.023), this finding was not significant after FDR (threshold level for adjusted FDR significance was 0.0028) (Figure 11).

	Collagenopathy (n=7) N (%)	PVI/PVHI without genetic findings (n=62) N (%)	Odds ratio	95% CI	P value
At the onset of pregnancy					
Maternal age, year, median [interquartile range]	25 [22-28]	28 [25-34]			0.089
Previous pregnancy loss	2/6 (33.3)	19 (30.6)	1.13	(0.09 - 8.7)	>0.99
Previous child death	1 (14.3)	3/61 (4.9)	3.2	(0.11 - 34)	0.36
Multiparity (>3 deliveries)	0 (0)	9 (14.5)	0.38	(0.02 - 7.1)	0.58
Conception by in vitro fertilization	0 (0)	3 (4.8)	0.88	(0.04 - 19)	>0.99
Twins	0 (0)	11 (17.7)	0.30	(0.02 - 5.6)	0.59
Preeclampsia	0 (0)	4 (6.5)	0.89	(0.04 - 18)	>0.99
Polyhydramnion	0 (0)	0 (0)	N/A	N/A	N/A
Oligohydramnion	0 (0)	5 (8.1)	0.70	(0.03 - 14)	>0.99
Delivery					
Placental abruption	1 (14.3)	8/61 (13.1)	1.10	(0.02 - 11)	>0.99
Chorioamnionitis (histological)	0 (0)	13 (21.0)	0.24	(0.01 - 4.6)	0.33
Emergency caesarean	2 (28.6)	18/61 (29.5)	0.96	(0.08 - 6.5)	>0.99
Apgar at the 5th min <7	0 (0)	14 (22.6)	0.22	(0.01 - 4.1)	0.33
Intubation at birth	0 (0)	17 (27.4)	0.17	(0.01 - 3.2)	0.18
Neonatal data					
Gender (boys)	1 (14.3)	33 (53.2)	0.15	(0.003 - 1.3)	0.11
Small for gestational age <10 percentiles	2 (28.6)	10/61 (16.4)	2.04	(0.25 - 13)	0.60
Head circumference <10 percentiles	3/5 (60)	5/46 (10.9)	12.3	(1.44 - 111)	0.023
Neonatal seizures	1 (14.3)	15/57 (26.3)	0.47	(0.01 - 4.4)	0.67
Artificial ventilation >24h	0 (0)	22 (35.5)	0.12	(0.01 - 2.2)	0.09

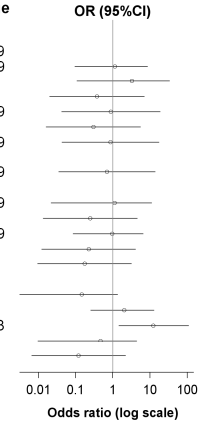

Figure 11. Prenatal and neonatal risk factors in children with collagenopathy (pathogenic/likely pathogenic variants in collagen genes) compared to children with PVHI/PVI without genetic variants. The adjusted false discovery rate significance threshold level is 0.0028 (OR, odds ratio; CI, confidence interval for the odds ratio; h, hours). (Ilves et al., 2023, published with permission from Sage Journals).

7.3.3. MRI characteristics of children with collagenopathy

Children with collagenopathies had significantly more often multiple MRI characteristics like: bilateral multifocal stroke (p=0.0029), bilateral severe white matter loss (p=0.01), bilateral hyperintensities in the white matter (p=0.008), moderate to severe enlargement of the lateral ventricles (p=0.01), extensive bilateral periventricular gliosis (p=0.016), lacunes in the contralesional hemisphere (in the putamen) (p=0.0098), moderate to severe decrease in the size of the ipsilesional caudate nucleus (p=0.018), putamen (p=0.0015) and thalamus

(p= 0.017) compared to children with PVI without genetic changes (Figure 12). CT images for clinical purpose were obtained only in 3/7 children with collagenopathies and all of them showed small bilateral parenchymal calcifications. MRI findings in children with perinatal stroke and with collagenopathy are presented in Figure 12.

	Collagenopathy (n=7) N (%)	PVI without genetic findings (n=62) N (%)	Odds ratio	95% CI	P value
MRI findings					
Unilateral stroke	3 (42.9)	49/61 (80.3)	0.18	(0.03 - 0.98)	0.048
Multifocal bilateral stroke	4 (57.1)	4/59 (6.8)	18.3	(2.76 - 116)	0.0029
Bilateral severe white matter loss	3 (42.9)	3/60 (5.0)	14.3	(1.84 - 98)	0.013
Bilateral T2W white matter hyperintensities	2 (28.6)	1/59 (1.7)	23.2	(1.42 - 682)	0.028
Enlargement of side ventricles (moderate, severe)	4 (57.1)	7/61 (11.5)	10.3	(1.77 - 59)	0.011
Extensive bilateral periventricular gliosis	4 (57.1)	8/60 (13.3)	8.7	(1.53 - 49)	0.016
Schizencephaly	1 (14.3)	3/59 (5.1)	3.1	(0.10 - 33)	0.37
Lacunes	2 (28.6)	0/59 (0)	54.1	(2.30 - 1274)	0.0098
Hemosiderin	4/5 (80)	25/35 (71.4)	1.6	(0.18 - 43)	>0.99
Thin corpus callosum	6 (85.7)	31/56 (55.4)	4.8	(0.63 - 114)	0.22
Decrease in size of nucleus caudatus (moderate, severe)	5 (71.4)	14/59 (23.7)	8.0	(1.34 - 60)	0.018
Decrease in size of putamen (moderate, severe)	5 (71.4)	7/59 (11.9)	18.6	(2.68- 143)	0.0015
Decrease in size of thalamus (moderate, severe)	5 (71.4)	14/60 (23.3)	8.2	(1.37 - 62)	0.017

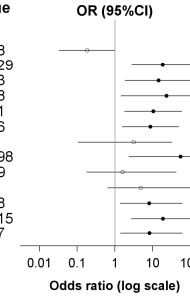

Figure 12. MRI findings in children with PVHI/PVI and collagenopathies compared to children without genetic changes. Only the raw p-values below the adjusted false discovery rate significance threshold of 0.035 are marked as significant (black dot in the figure). (OR, odds ratio; CI, confidence interval for the odds ratio; h, hours). (Ilves et al., 2023, published with permission from Sage Journals).

7.3.4. Characteristics of outcome in children with possibly stroke-causing genetic variants

No children died in the group of collagenopathies or other pathogenic variants during the follow-up period (Table 6). Children with pathogenic variants in collagen genes had more often moderate to severe motor deficit (6/7, 85.7%) compared to children without genetic variants (12/78, 20.7%), respectively**,** **(**OR, 233 [95% CI, 2.8–531], p=0.0013). Children with pathogenic variants in collagen genes developed also epilepsy more often compared to children without genetic variants (57.1% versus 15.5%, respectively, (OR, 7.3 [95% CI, 1.3–41], p=0.025).

Table 6. Neurodevelopmental outcome in children with PVHI/PVI: with variants in collagen genes, with variants in other stroke related genes and without pathogenic variants in stroke related genes.

	Total N (%)	Pathogenic variants in collagen genes N (%)	Pathogenic variants in other stroke related genes N (%)	Without genetic variants N (%)
Moderate-severe motor deficit	20/69 (27.5)	6/7 (85.7)**	2/4 (50)	12/58 (20.7)
Epilepsy	14/69 (20.3)	4/7 (57.1)*	1/4 (25)	9/58 (15.5)
Recurrent hemorrhage	1/73 (1.4)	1/7 (14.3)	0 (0)	0 (0)
Death	2/73 (2.7)	0/7 (0)	0/4 (0)	2/62 (3.2)
Total	73 (100)	7 (100)	4 (100)	62 (100)

*p<.05, **p<.01 collagenopathy versus no genetic variants

8. DISCUSSION

This study provided new insights into the etiology and time of development of PVHI and PVI. It demonstrated that maternal bacterial infection, especially pyelonephritis, is an important risk factor for PVHI and PVI.

Up to now, studies on the mechanism of development of PVHI in preterm born children have focused on birth distress, postnatal environment and changes in the cerebral blood flow, which can lead to rupture of the fragile germinal matrix vessels in very premature infants ("Volpe's Neurology of the Newborn – 6th Edition," n.d.). Strategies aimed to alleviate previously thought causes, reducing the risks related to delivery and intensive care, have not led to a decrease in the incidence of PVHI.

As no clear etiology of presumed antenatal stroke has been identified during pregnancy, then no timely measures have been taken or can be taken to decrease the number of cases of presumed antenatal PVI and later lifelong disability. Yet without the knowledge of risk factors, prevention of stroke development is impossible.

8.1. Prenatal etiology of stroke (Substudies I, II)

According to the current study, there are certain etiological factors during pregnancy, the avoidance of which or prompt treatment could reduce the incidence of antenatal or presumed antenatal PVI /PVHI.

8.1.1. Risk factors at the onset of pregnancy

The mothers of children with presumed antenatal PVI or PVHI and those of the control group showed no statistical differences in the following risk factors before pregnancy: maternal age, number of deliveries, mode of conception, rate of prenatal hypertension or diabetes, and smoking and drinking habits. Nor did preterm born children with antenatal onset or neonatal onset of PVHI and controls show differences in potential maternal risk factors prior to pregnancy.

Although the rate of twin pregnancies was not different between the control group and the group of presumed antenatal PVI, there were significantly more twin pregnancies among the mothers of children with PVHI compared to the mothers of infants with presumed antenatal PVI. Multiple logistic regression analysis in this study showed that twin pregnancy and previous spontaneus pregnancy loss were independent predictors for development of PVHI in preterm infants compared to presumed antenatal PVI in term born children. Twin pregnancy is a major risk for preterm delivery: twins account for 3.2% of all live births, but they account for 20% of all preterm deliveries, with 10.7 % of twins delivered before GW 32 (Roman et al., 2022). Twin pregnancies have 5 times higher risk of early neonatal and infant death related to prematurity (Roman et al., 2022); monochorionic pregnancies are at a particular risk (Mackie et al., 2016).

The mothers of the neonates in the groups of antenatal, unknown and neonatal onset of PVHI or severe IVH were of similar age, had a similar number of previous pregnancies, deliveries, threatened pregnancy losses and imminent preterm deliveries, and had a similar number of twin pregnancies.

8.1.2. Risk factors during pregnancy

Infection was the only risk factor during the pregnancy that was significantly different in the group of presumed antenatal PVI and the control group. According to multiple logistic analysis, preeclampsia, GTI and other bacterial infections were also independent predictors of presumed antenatal PVI compared to control.

Bacterial infection treated with antibiotics was the only risk factor during pregnancy that was significantly different for preterm born children with antenatal onset and those with neonatal onset of PVHI.

8.1.3. Maternal infection as a cause of antenatal onset PVHI and presumed antenatal PVI

This study is the first to demonstrate maternal bacterial infection and chorioamnionitis to be risk factors leading to antenatal development of PVHI in preterm born children compared to children with neonatal onset of PVHI. In case the status of the fetus improves and preterm birth is not the primary outcome of infection, pregnancy continues until term birth and PVI will be diagnosed later in infancy as presumed antenatal PVI.

The rate of prenatal bacterial infection was higher among term born children with presumed antenatal PVI compared to the control group of term born children, as well as among preterm born children with antenatal development of PVHI compared to neonatal development of PVHI. Furthermore, our study is the first to show that the time of maternal bacterial infection was similar in the groups of presumed antenatal PVI in term born children and PVHI in preterm born children. It means that infection occurring at the same gestational age indicates the uniform stage of vascular development with a prominent germinal matrix between GW 21–31.

The findings of the study demonstrated that GTI, including bacteriuria, accounted for at least 60% of all infections during pregnancy in term born controls, in infants with presumed antenatal PVI and also in preterm infants with PVHI. However, 38% of the mothers of the control group were diagnosed with asymptomatic bacteriuria and recieved antibacterial therapy, while no such cases were seen in the stroke groups. Infection in the control group was treated in the first half of pregnancy, while the mothers of both children with presumed antenatal PVI and those with PVHI had infection between GW 21–31.

In contrast, pyelonephritis developed rarely among the mothers of the control group, but was found in 50% of the infection cases in the group of presumed antenatal PVI. There were no differences in the proportion of pyelo-

nephritis between the group of presumed antenatal PVI and the group of PVHI, indicating that a similar mechanism may have played a role in the development of stroke in both groups.

Fetal inflammation has emerged as a major mechanism of the disease responsible for complications in the perinatal period, gene-environment interactions can also be determinants of the likelihood of preterm labor and delivery and probably the risk of fetal injury (Romero et al., 2007). The persistence of relationship between umbilical cord inflammation and elevated concentrations of inflammation-related proteins in the blood on postnatal day 7 suggest the existence of phenomena that contribute to a sustained systemic inflammation during the postnatal period (Leviton et al., 2011).

In a metaanalysis, clinical and histological chorioamnionitis was significantly associated with cerebral palsy in both preterm and term born children (Wu and Colford, 2000). The relative risk of disability in vision, hearing, speech and motor development was higher among preterm born children with histological chorioamnionitis than among those with non-histological chorioamnionitis (Suppiej et al., 2009). In the present study the rate of clinical and histological chorioamnionitis was higher among children with antenatal development of PVHI compared to neonatal development of PVHI; however, maternal chorioamnionitis at birth was not different between groups of children with presumed antenatal PVI and controls.

In multiple regression analysis maternal bacterial infection was an independent predictor of presumed antenatal PVI in term born children compared to controls, as well as for antenatal onset of PVHI compared to neonatal onset of PVHI in preterm born infants. These findings support indication for fetal cerebral US within a few weeks after serious bacterial infection during pregnancy. When the child is born, early neonatal US is recommended to evaluate possible brain damage following maternal bacterial infection. Children with presumed antenatal PVI as well as preterm born children with antenatal onset PVHI may not present any clinically detectable neurological symptoms during the neonatal period after birth. However, early detection of brain damage facilitates immediate rehabilitation. The awareness of the need for prompt treatment of bacterial infection is crucial among all specialists in charge of pregnant women.

Urinary tract infection is a very common bacterial infection during pregnancy. A reduction in the incidence of pyelonephritis was attained when screening programs were introduced at a large teaching hospital in Spain (Gratacós et al., 1994). If asymptomatic bacteriuria is untreated, women frequently develop symptomatic or recurrent urinary tract infection (Kazemier et al., 2015) and mothers with untreated asymptomatic bacteriuria had up to 20–30% risk for developing pyelonephritis later in pregnancy (Nicolle, 2015).

A study with 219 612 singleton deliveries, including 165 cases with hospital admission due to acute pyelonephritis, found significantly more preterm deliveries at GW <37, overall earlier gestational age, intrauterine growth restriction and lower birth-weight infants, as well as other infectious morbidities such as

urinary tract infection and pneumonia, and also significantly higher rates of labor induction, suspected fetal distress, placental abruption, and lower Apgar scores at 1 minute (Farkash et al., 2012). Acute antepartum pyelonephritis was found to be an independent risk factor for preterm delivery (OR, 2.6 [95% CI, 1.7–3.9], p<0.001) (Farkash et al., 2012). In the present study the odds ratio for developing presumed antenatal PVI was 50 times higher in the children of mothers with pyelonephritis compared to children of mothers without bacterial infection (OR, 50 [95% CI, 18–133], p=0.001),

Many studies have found an increased prevalence of adverse pregnacy outcomes after maternal **pneumonia** occurring during pregnancy, however, pneumonia during pregnancy is a rare condition. In the present study, there was only one case of pneumonia in the control group and one among the mothers of children with PVHI. According to some studies, the odds ratio for preterm births (OR 1.71; 95% CI 1.42–2.05), lower birth weight, higher rate of caesarean section, lower Apgar score and preeclampsia/eclampsia is higher for women with pneumonia compared to women without pneumonia (Romanyuk et al., 2011; Chen et al., 2012). Furthermore, another study found pneumonia during pregnacy to be an independent risk factor, among others, also for placental abruption (OR 4.2; 95% CI 1.9–9.1) (Romanyuk et al., 2011).

There was also a case among children in the group of presumed antenatal PVI whose mother had **appendicitis** during pregnancy at the age of 31GW, which has not been reported earlier as a possible risk factor for presumed antenatal PVI.

Women with appendicitis showed higher rate of pregnancy loss at GW <20 in a study with 127 monitored women. Appendicitis diagnosed in the first trimester was associated with an increased risk of pregnancy loss at GW <24 (Theilen et al., 2017). After adjusting for covariates, pregnant mothers with acute appendicitis were twice as likely as those without acute appendicitis to suffer fetal loss (OR 2.05; 95% CI 1.85–2.28) (Dongarwar et al., 2020).

The rate of complications is significantly increased in the case of perforated appendix compared to non-perforated appendix (52% vs. 17%) (p=0.013) and fetal death occurred in 4 cases (8%) only in the group of perforated appendix (Yilmaz et al., 2007). Compared to pregnant women without the diagnosis of acute appendicitis, there was a statistically significant and an almost fivefold increase in inpatient death among those diagnosed with acute appendicitis (Dongarwar et al., 2020).

Although the overall incidence of appendicitis during pregnancy was not increased in a study by Tamir et al., with higher frequency in the second trimester, the diagnosis was more difficult to establish. Prompt diagnosis is important as delay in treatment over 24 hours increases the risk of appendicular perforation, which occurred in 23 (43%) of 54 patients with proven acute appendicitis, with symptoms over 24 hours (p<0.0005) (Tamir et al., 1990). Another study supporting the significance of the time of initiating treatment found the interval between onset of symptoms and operation to be an indepen-

dent risk factor for perforation (OR 1.095; 95% CI 1.035–1.158; p=0.002), with a cut-off value of 20 h in estimating the risk of perforation (Yilmaz et al., 2007).

8.1.4. Risk factors during delivery and postnatal complications

Children with presumed antenatal PVI showed no differences, compared to the control group, regarding delivery risk factors: mode of delivery, need for stimulation, fetal dystocia during delivery, placental abruption, chorioamnionitis during delivery and signs indicating acute distress during delivery, as well as Apgar score and need for resuscitation (Figure 2 and Table 1). The median 5 minute Apgar score was even higher in the group of presumed antenatal PVI (p=0.0236) compared to the control group (Table 1). This indicates that children with antenatal presumed PVI are stable at birth and do not show any specific clinical signs.

Similarly, preterm children with antenatal or neonatal onset of PVHI showed no differences in Apgar score, birth weight or head circumference at birth. However, preterm born children with neonatal development of PVHI showed more often distress at delivery leading to emergency caesarean section, compared to children with antenatal onset of PVHI. Postnatal intensive care for children with neonatal onset of PVHI was complicated: surfactant, cardiotonic-vasoactive support, medical or surgical treatment of persistent arterial duct and long term artificial ventilation were more often needed. Multiple regression analysis showed that surfactant treatment was an independent risk factor for development of neonatal onset versus antenatal onset PVHI. In children with neonatal development of PVHI, the likely causes leading to stroke are asphyxia, changes in the cerebral blood flow, and perinatal hypoxic events as the earlier known major risk factors for PVHI ("Volpe's Neurology of the Newborn – 6th Edition," n.d.). The development of PVHI was associated with emergency caesarean section in 30%, patent arterial duct in 19%, thrombocytopenia in 18%, pneumothorax in 12%, seizures in 11%, intrauterine growth restriction and/or preeclampsia in 8% of cases in preterm infants with PVHI (Bassan et al., 2006b; Roze et al., 2008; Dudink et al., 2008; Soltirovska Salamon et al., 2014; Roy et al., 2022). However, the etiology of PVHI in preterm infants is thought to be not fully understood even after a recent systematic review (Roy et al., 2022).

8.1.5. Neurological symptoms of PVHI/PVI after birth

Development of severe IVH/PVHI leads to a catastrophic syndrome with a dramatic presentation of neurological symptoms in preterm born children. Although the postnatal development of IVH/PVHI leads to disastrous deterioration in the child's status, interestingly, 25–50% of preterm born children with IVH/PVHI may fail to reveal any symptoms, indicating that the lesion or the neurological signs of PVHI may be so subtle that they are overlooked (Dubowitz et al., 1981; "Volpe's Neurology of the Newborn – 6th Edition," n.d.); no

explanation for this has been proposed. However, monitoring in intensive care units has improved after these earlier studies, which may have decreased the number of silent cases. According to a recent review, stroke in preterm infants is unlikely to present with focal seizures and may be asymptomatic or have non-specific clinical presentation (Roy et al., 2022). In the present study, 27/120 (22.5%) cases of severe IVH/PVHI were considered antenatal in origin. Children with antenatal onset IVH/PVHI had significantly less neonatal seizures or need for intubation after birth. It can be suggested that at least some of these infants with antenatal development of IVH/PVHI have already become stable by birth and might have mild neurological symptoms after birth.

Children with antenatal compared to neonatal development of PVHI, are likely to have different genesis and time of stroke as some of the children with antenatal PVHI have mild symptoms at birth without the need for intubation and need for surfactant treatment, as well as low rate of seizures. The risk factor profile differed significantly between the groups of antenatal and neonatal PVHI: the group of antenatal onset showed significantly higher prevalence of maternal bacterial infections than the group of neonatal onset. The neonates of the group of antenatal onset and the group of unknown time-onset, compared to the group of neonatal onset, had significantly less risk factors related to fetal or neonatal stress and complications, inclucing emergency caesarean section, need for cardiotonic/vasoactive treatment and artificial ventilation >24h.

The finding of scarce neonatal symptoms in preterm born children with antenatal development of PVHI is similar to the finding in term born children with presumed antenatal PVI (Ilves et al., 2016). The children of both groups, with the antenatal onset of PVHI and as well with presumed antenatal PVI, have probably stabilized by birth after the antenatal onset of stroke. Maternal bacterial infection is a possible cause of development of antenatal stroke at least in some cases. As children with antenatal development of PVHI/PVI can be symptom free after birth, early cerebral US may detect brain damage in children whose mother had infection during pregnancy.

8.2. Genetic causes of antenatal stroke (Substudy III)

Besides environmental factors during pregnancy, distress at birth, or fluctuations in the cerebral blood flow, as well as genetic factors may also be important in development of severe intraventricular hemorrhage and PVHI/PVI in fetuses and neonates (Ment et al., 2014, 2015).

This study shows high rate (11/85; 13%) of pathogenic/likely pathogenic gene variants causing collagenopathies, coagulopathies and other genetic variants associated with microangiopathy and stroke in children with PVHI/PVI. The gene variants causing collagenopathies were the most frequently detected gene variants among children with PVHI/PVI. Earlier, a similarly high prevalence (16%) of *COL4A1* variants has been described in patients with porencephaly (Yoneda et al., 2013).

8.2.1. Collagenopathies

According to this study, both term born children with antenatal and presumed antenatal PVI and preterm born children with PVHI show high prevalence of collagenopathies, with a high number (7/11) of pathogenic/likely pathogenic variants in *COL4A1/2* and *COL5A1* genes.

8.2.1.1. Type IV collagenopathies

Among term born children with antenatal or presumed antenatal PVI of various severity, type IV collagenopathy is often underdiagnosed due to its variable expressivity in these genetic variants. Among children with PVHI/PVI, *COL4A1/A2* variants were most often detected among pathogenic variants. Antenatal and neonatal hemorrhage, schizencephaly and porencephaly (Mancini et al., 2004; Knaap et al., 2006; Mineyko and Kirton, 2011; Verbeek et al., 2012; Yoneda et al., 2012; Matsumoto et al., 2015; Khalid et al., 2018; Zagaglia et al., 2018; Cavaliere et al., 2021; Hausman-Kedem et al., 2021b; Itai et al., 2021; Maurice et al., 2021), as well as brain developmental disturbances like heterotopias and polymicrogyria have been previously associated with type IV collagenopathy (Hausman-Kedem et al., 2021a).

Earlier, 24 splicing mutations, 4 pathogenic small deletions and 5 small insertions are described as pathogenic in the HGMDPro database. The total allele frequency of loss-of-function variants and of the variants listed as (likely) pathogenic in the ClinVar database for *COL4A1* is 0.00302. In the general population's database, the gnomAD v2.1.1 a carrier prevalence of pathogenic varinats of *COL4A1* is 1 per 1650, however, in the present study children with PVHI/PVI had collagenopathy in 1:12 cases.

Children with type IV collagenopathies have shown high prevalence of microbleeds, cerebral white matter abnormalities and lacunar infarcts, indicating the shared pathogenesis of microangiopathy (Knaap et al., 2006; Maurice et al., 2021). Cerebral lesions can be severe and/or multifocal ischemic/hemorrhagic and of different ages with associated schizencephaly and porencephaly (Maurice et al., 2021).

Different severities of clinical and radiological findings are typical of type IV collagenopathies, which makes it difficult to distinguish them from findings in children presenting no changes in collagen genes and leads to the underdiagnosing of collagenopathies. This study demonstrates a case when initially mild presentation can progress to early adulthood intracranial macrohemorrhage with poor outcome. In other ischemic types of perinatal stroke the risk for recurrence is low (Lehman et al., 2017).

8.2.1.2. Type V and I collagenopathies

This study is the first to present a premature child with the pathogenic *COL5A1* variant with severe intraventricular hemorrhage and PVHI. In addition, a variant of unknown significance was identified in *COL5A1* gene in three children in

COL1A1 gene and in three children. The classical type Ehlers-Danlos syndrome develops in patients with type V collagenopathies and the classical, vascular or arthrochalasia type Ehlers-Danlos syndrome develops in type I collagenopathies. The Ehlers-Danlos syndrome comprises a clinically and genetically heterogeneous group of heritable connective tissue disorders with the main features being skin hyperextensibility, joint hypermobility, tissue fragility and easy bruising (Hamel, 2019). In the Ehlers-Danlos syndrome, bleeding diathesis is caused by an abnormal capillary structure due to deficiency of normal perivascular collagen, leading to poor support for blood vessels which can easily rupture. Specific gene variants of *COL1A1* can lead to arterial rupture or dissection <40 years of age (Hamel, 2019). *COL5A1* is one of the genes which has been associated with predisposition to preterm birth earlier (Anum et al., 2009). More studies are needed to evaluate the *COL5A1* and *COL1A1* variants in relation to development of PVHI and PVI. In the present study the child with the *COL5A1* variant had bilateral multifocal strokes with early onset epilepsy and poor neurodevelopmental outcome, the gentic variant was inherited from the father. Like in children with *COL4A1* variants, the clinical finding of *COL5A1* can be heterogenous and genetic testing is needed even in mild cases of PVHI/PVI.

8.2.1.3. Collagenopathy and time of development of PVHI/PVI.

This study supports the earlier idea that in some PVHI/PVI cases genetic predisposition like connective tissue disorders might be the leading factor for development of IVH (Yoneda et al., 2012, 2013; Ment et al., 2014; Kirkham et al., 2018; Zagaglia et al., 2018; Maurice et al., 2021).

The *COL4A1/A2* and *COL5A1* pathogenic variants were prevalent in children with antenatal and presumed antenatal PVI/PVHI and also in preterm born children with PVHI. This finding can be explained by the period when PVHI/PVI occurs between GW 24 and 34 (Dunbar and Kirton, 2019; Fluss et al., 2019; eurUS.brain group et al., 2020), as shown also in previous studies focusing on antenatal IVH and PVHI cases (Özduman et al., 2004; Elchalal et al., 2005). The median age of the diagnosis of fetal hemorrhage was as early as GW 24 (22–26) in fetuses with *COL4A1/A2* pathogenic variants and later during gestation GW 32 (29.5–34.5) in fetuses without genetic changes (Maurice et al., 2021). Presumably, disruption of the immature fragile vessels and hemorrhage in the germinal matrix is more likely to occur with pathogenic changes in *COL4A1*/*A2* or *COL5A1* genes.

8.2.1.4. Other risk factors in children with collagenopathy

No pregnancy, delivery or neonatal symptoms can distinguish children with PVHI/PVI and collagenopathy from those without an identified genetic pathogenic variant, as described also earlier (Zagaglia et al., 2018). If antenatally developed IVH/PVHI is not detected with antenatal US and the pregnancy

continues till term delivery, the delivery is likely to be uneventful as in children with presumed antenatal PVI. However, this study found that the head circumference of 3/7 children in this group with collagenopathy was <10 percentiles, all of them with severe brain damage with bilateral multiple stroke, white matter loss, and poor outcome with severe motor deficit. A small head circumference at birth can be a possible sign of severe antenatal brain damage, including collagenopathy and PVHI. As suggested earlier, collagenopathy, which has caused antenatal stroke, should be one of the differential diagnoses in children with microcephaly at birth (Yoneda et al., 2013; Zagaglia et al., 2018).

8.2.1.5. MRI findings in children with collagenopathy

MRI findings in children with collagenopathy are diverse. The results of this study coincide with those of earlier studies that report high prevalence of cerebral white matter abnormalities, indicating microangiopathy, leading to development of porencephaly and/or schizencephaly in children with collagenopathy (Vilain et al., 2002; Knaap et al., 2006; Verbeek et al., 2012; Yoneda et al., 2013; Meuwissen et al., 2015; Zagaglia et al., 2018; Hausman-Kedem et al., 2021a; Maurice et al., 2021). *COL4A1* variants can compromise the structural integrity of the vascular basal membrane, causing capillaries to have interruptions in their basement membranes with normal and markedly increased thickness (Knaap et al., 2006; Maurice et al., 2021). Changes in the basement membrane of vessels cause high prevalence of microbleeds, cerebral white matter abnormalities and lacunar infarcts similarly to other microangiopaties (Knaap et al., 2006; Maurice et al., 2021). In the present study the radiological findings of collagenopathies show a wide spectrum of severity, as has been reported earlier (Knaap et al., 2006; Yoneda et al., 2013; Meuwissen et al., 2015; Zagaglia et al., 2018; Itai et al., 2021). Some of the children have severe and/or multifocal ischemic/hemorrhagic cerebral lesions of different ages, which are associated with schizencephaly and porencephaly (Maurice et al., 2021). Vascular disruption before GW 24 results in schizencephaly and after GW 24 in porencephaly (Hino-Fukuyo et al., 2016).

However, some children with collagenopathies show a unilateral porencephalic enlargement of the lateral ventricle and/or periventricular white matter damage (Knaap et al., 2006; Yoneda et al., 2013; Maurice et al., 2021), like most children with PVI/PVHI (Bassan et al., 2006a, 2007; eurUS.brain group et al., 2020). In this study, compared to children with PVI/PVHI without genetic changes, children with collagenopathy had significantly more often multifocal bilateral stroke, extensive bilateral periventricular gliosis, moderate to severe irregular enlargement of the lateral ventricles, as well as a moderate to severe decrease in the size of the caudate nucleus, putamen, and thalami. However, these findings are unspecific. More specific findings like extensive bilateral diffuse white matter T2 weighted signal hyperintensities and extensive bilateral white matter loss and lacunes on MRI were noted more often in children with

collagenopathies, indicating microangiopathy, as has been found earlier (Zagaglia et al., 2018). However these findings were not obtained in all cases.

Hemosiderin, which is often found in the lateral ventricles, in the periventricular area and/or in the parenchyma, as well as schizencephaly and thin corpus callosum, were not different between children with and without genetic variants. Bilateral parenchymal calcifications were found in all three children with collagenopathies for whom CT was available; a similar finding has been reported earlier (Yoneda et al., 2013; Itai et al., 2021; Maurice et al., 2021). However CT was not available in all cases. Therefore, in the case of cerebral calcifications, collagenopathies should be considered as a differential diagnosis, besides congenital infections.

8.2.1.6. Outcome in children with collagenopathy

In conjunction with variable severity of radiological findings, the clinical outcome and severity of disability in children with collagenopathy are also variable. Severe motor deficit and epilepsy were found significantly more often in children with pathogenic variants in collagen genes (85.7%) compared to children without genetic variants (20.7%). Earlier, a wide spectrum of phenotypes has been reported for patients with collagenopathy, including congenital hemiparesis, epilepsy and intellectual disability (Meuwissen et al., 2015; Zagaglia et al., 2018; Hausman-Kedem et al., 2021a; Itai et al., 2021). Epilepsy developed in 4 of 7 patients with the *COL4A1/A2* and *COL5A1* pathogenic variants in our study. A larger multicenter study found epilepsy in 55/123 patients with the *COL4A1/2* pathogenic variants (Zagaglia et al., 2018). One patient in our study with collagenopathy developed a spontaneous intracranial macrohemorrhage later in life, which significantly worsened the already poor outcome; similar data has been reported also earlier (Knaap et al., 2006). Considering that the cohorts of the present study comprise patients born since 1994, it is possible that further macrohemorrhage events can occur as the patients get older.

8.2.2. Coagulopathies

The role of coagulopathies in the pathogenesis of perinatal stroke is still controversial. This study identified only a few patients with genetic variants in the genes leading to coagulopathies among children with PVHI/PVI. Compared to the number of patients with collagenopathies, the number of patients with changes in the genes causing coagulopathies was lower. Based on these findings, genetic testing should be suggested for patients with PVI, first of all to evaluate collagenopathies, but also the genes related to coagulopathies should be added to analysis.

Contrary to this study, an earlier study of preterm infants with an atypical presentation of PVHI recommends testing for thrombophilia, especially for the factor V Leiden and prothrombin gene mutation which were found in 7/17

cases, although the variants of *COL4A1* gene were also found in 2/17 cases (Harteman et al., 2012).

In support of the present study, Kirkham and co-authors emphasized the role of arterial endothelial dysfunction rather than thrombocytopenia, and maternal and fetal clotting disorders (Kirkham et al., 2018).

The prothrombin (F2) 20210G>A [rs1799963 G>A] genetic variant and the factor V Leiden mutation predispose to inherited thrombophilia, their prevalence is high in the Caucasian population (Harteman et al., 2012). In Estonia the prevalence of the carriers of the prothrombin common variant was 3.3% and the prevalence of the carriers of the factor V Leiden variant was 3% among 400 tested controls (Laugesaar et al., 2010).

In this study there were no patients with the factor V Leiden variant among children with PVHI/PVI, however, one of them (1/85, 1.2%) with presumed antenatal PVI had the prothrombin 20210G>A (rs1799963) variant. One of the preterm born children with PVHI had antithrombin 3 deficiency due to the *SERPINC1* variant inherited from the father.

A review of articles on the genetic profiling of antenatal intracranial hemorrhage found two case reports on factor V deficiency, but none on the prothrombin 20210G>A variant (Cavaliere et al., 2021). Although this study did not note an increased prevalence of the factor V Leiden and prothrombin 20210G>A gene variants, predisposition to stroke in the case of these genetic variants can not be ruled out.

8.2.3. Other gene variants predisposing to stroke

This study found two familial cases with variants in the genes that can be associated with microangiopathy and stroke or stroke-like episodes. The most common mitochondrial variant m.3243A>G in *MT-TL1* was detected in a preterm born child with PVHI, as well as in her mother and in the other family members. The family history included hearing loss, diabetes and migraine. However, the link between the clinical findings of this study and mitochondrial DNA variation in the MELAS (Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like Episodes) syndrome is uncertain. The relatively small size of infarction in this patient adds to the uncertainity of causality in this case. However, the MELAS syndrome is associated with microvascular blood perfusion damage and stroke-like episodes at the age under 40 years with serious long-term consequences (Cheng et al., 2022) and can not hence be ignored.

The *NOTCH3* pathogenic variant in the group of presumed antenatal PVI was found in one child and in his father. Pathogenic variants in *NOTH3* are known to cause CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy). The child had focal porencephaly and severe motor deficit, but no other signs of microangiopathy by the age of nine years. Changes in *NOTCH3* lead to cerebral autosomal dominant

arteriopathy with subcortical infarcts and leukoencephalopathy, including white matter hyperintensities, lacunar infarcts and microbleeds (Stojanov et al., 2015).

A previous report described the prenatal diagnosis of the NOTCH variant in the fetus of a couple where the father had a known gene variant (Milunsky et al., 2005); however, the finding has not been reported for children with perinatal stroke earlier.

Although neither of these genes and variants have been reported in children with perinatal stroke, we cannot ignore these findings and further research is needed in this field.

8.2.4. Indications for genetic testing

Today, routine genetic evaluation of all patients with PVHI/PVI is not yet common clinical practice. Earlier, mostly coagulopathies were tested for in children with PVHI/PVI, but data about the role of inherited thrombophilia in the pathogenesis of perinatal stroke is controversial. Some studies have suggested abnormalities in coagulation as a possible cause of perinatal stroke (Mineyko and Kirton, 2011; Cavaliere et al., 2021), while others have found that the thrombophilia risk is not increased in term born children with perinatal stroke, including patients with presumed antenatal PVI, compared to controls (Laugesaar et al., 2010; Curtis et al., 2017). Systematic genetic testing has been suggested earlier for term born children with intraparenchymal hemorrhages (Maurice et al., 2021), for children with severe antenatal intracranial hemorrhage (Cavaliere et al., 2021), and for children with porencephaly (Yoneda et al., 2013). This study agrees with the earlier suggestion by Kirkham and coworkers (Kirkham et al., 2018) that arterial endothelial dysfunction is of greater consequence than thrombocytopenia in children with PVHI/PVI.

PVHI/PVI leads to lifelong motor deficit and cognitive impairment, epilepsy, behavioral problems and academic difficulties (Kirton and deVeber, 2013; Dunbar and Kirton, 2018; Laugesaar et al., 2018; Lõo et al., 2018; Dunbar and Kirton, 2019). Poor neurodevelopmental outcome has been found in preterm survivors with PVHI (Bassan et al., 2007). We found that although children with the most severe collagenopathy had been diagnosed already clinically, five of 11 children with pathogenic/likely pathogenic variants of collagenopathy had not been tested earlier genetically, among them three children with collagenopathy, as they had been considered too mild cases clinically and/or radiologically.

Delivery related and postnatal complications are mostly associated with PVHI ("Volpe's Neurology of the Newborn – 6th Edition," n.d.); therefore, the awareness of the genetic causes of PVHI/PVI gives the children's parents the understanding that not only preterm birth by itself, but also perinatal stress and possible treatment failure can be the underlying causes of severe PVHI (Ment et al., 2014, 2015). In all children with PVHI/PVI, both term born and preterm, genetic testing should be considered, and collagenopathy, including *COL4A1/A2* and *COL5A1/A2*, needs the closest attention. As there might be also other

gene variants leading to perinatal stroke, a large gene panel or exome sequencing should be preferred. Genetic testing should lead to counseling of the family for recurrence risks in further pregnancies and counseling of the patient for the threat of later macrohemorrhages in early adulthood. Genetic testing shoud be offered even in mild cases.

8.3. Limitations

8.3.1. Data collection

The pregnancy and delivery risk factors for children with PVI/PVHI were collected retrospectively from different hospitals. To reduce a possible bias, standardized definitions of the risk factors were used to collect data from medical records of the hospitals where the children with stroke were born. Since no data of bacteriuria screening was available for earlier cases, because it was introduced in Estonia in 2011, this study may have underestimated the true prevalence of infections in PVHI/PVI patients.

8.3.2. Group size

The study groups were small due to the low prevalence of perinatal stroke. Heterogeneity in combination with low prevalence makes standard group average-based statistics difficult to apply as reported earlier (Kirton et al., 2021). Some cases of term born children with mild hemiparesis with presumed antenatal PVI may have been missed, however, the problem of underdiagnosing presumed PVI is global (Özduman et al., 2004). On the other hand, our study is the largest study of patients with presumed antenatal PVI, who were evaluated genetically and whose data of neurodevelopmental outcome and follow-up MRI were available.

8.3.3. Length of the study period

The study period was long including the time when presumed perinatal stroke was not a widely recognized entity, however, the prospective collection of patients in EPSD started in 2004. To compensate for the long study period, the data was adjusted for the year of birth where applicable.

During the study period treatment strategies for preterm delivery changed: transport of high risk pregnant women for delivery at higher level hospitals, screening of mothers for asymptomatic bacteriuria, prenatal steroid and magnesium administration and changes in postnatal treatment like surfactant administration in preterm infants (Handley et al., 2018). In addition, the socio-economic standard in the country improved during the long study period. Still, the risk factors prior to and during pregnancy remained largely unchanged. A possible bias in the risk factors was compensated for with adjustment of the data of preterm neonates for the year of birth and gestational age.

8.3.4. Incomplete data about infection

The true prevalence of infections in the PVHI/PVI groups could have been underestimated due to the poor documentation of antibiotic treatment or bacteriuria screening in the mothers of children with perinatal stroke. However, although the data of all antibiotics prescribed for and purchased by the mothers of the control group during pregnancy was available at the prescription center, there were still significantly less mothers with infection in the control group compared to the groups of perinatal stroke.

8.3.5. Recognition of PVHI/PVI

The presumed mechanism of periventricular porencephaly is venous medullary infarction. While the findings in PVHI are specific (Takanashi et al., 2003; Kirton et al., 2008), its radiological outcome can be similar to that of prenatal distal perforator artery stroke, which can not be easily discriminated (Govaert, 2009).

Additionally, there is a previously published case of a boy born at GW 23 who developed postnatal PVHI which led to schizencephaly in this study group (Ilves et al., 2020). There can be other cases of schizencephaly or other cortical anomalies that are diagnosed as cortical malformations but not stroke. In some cases schizencephaly probably develops after stroke prior to GW 24, before neuronal migration is completed.

8.3.6. Genetic evaluation

Genetic testing in the group of deceased preterm born children was limited by only two pretem born children with PVHI, in whom the samples for genetic testing were taken prior to death.

As preterm children with the worst outcome (death) could not be tested, the prevalence of genetic variants among the preterm population with severe IVH or PVHI can be underestimated.

8.3.7. Outcome

It was impossible to retrospectively evaluate the effect and availability of rehabilitation for children with PVHI/PVI. Early rehabilitation, different interventions, and caregivers' motivation can affect cognitive and motor outcome in children with perinatal stroke, and can create an unknown bias in neurodevelopmental outcome. However, the postnatal care and availability of rehabilitation for children with genetic variants and without any genetic variants were similar.

8.4. Contribution to the field

Children with perinatal stroke are at a high risk for permanent lifelong impairment. It is very difficult to treat stroke patients, as rehabilitation only alleviates their condition, but can not fully treat the underlying brain damage. To avoid lifelong impairment and to improve the quality of life of patients with stroke and their families, as well as to reduce the social and economic burden to the society, prevention of stroke is the best option. The knowledge of etiology and risk factors is the prerequisite for disease prevention. The knowledge of the cause of stroke can mitigate depression and the feeling of guilt among parents.

This research demonstrates that prenatal bacterial infection, especially during the second half of pregnancy, may be an important risk factor for not only fetal death, imminent pregnancy loss, and preterm delivery, which are known earlier, but also for development of antenatal PVI in term born children and antenatal PVHI in preterm born children. This study supports the notion that the germinal matrix, rich in vulnerable unmaturated blood vessels between GW 24–34, is susceptible to bleed and may cause a specific antenatal vascular type of stroke -PVHI/PVI.

Pregnant women are screened and treated for asymptomatic infection in the first half of pregnancy. This study shows that bacterial infections are less frequent in the second half of pregnancy. It also demonstrates that if a bacterial infection occurs during the second half of pregnancy, it can lead to fetal brain damage and antenatal stroke. In the group of children with PVHI/PVI, two thirds of maternal infections during pregnancy were GTI. However, also other maternal infections can lead to fetal stroke and require prompt treatment. Hence, active measures should be taken to prevent and immediately treat maternal bacterial infections, among them asymptomatic bacteriuria, to avoid the inflammatory reaction of the fetus.

The awareness of potential infection related risks should be raised among obstetricians, but also among other specialists like emergency department's specialists, and general practitioners who regularly treat pregnant women.

Children from pregnancies complicated with pyelonephritis or other bacterial infections may require neurological follow-up. Fetal US or early neonatal US is indicated in cases of maternal bacterial infection during the second half of pregnancy to screen for possible stroke and to start early rehabilitation. Early MRI is needed if hemiparesis is detected in children with maternal infection, as early diagnosis facilitates the timely rehabilitation of patients with perinatal stroke.

Genetic etiology is important in children with perinatal stroke. The findings of the research indicate that collagenopathies, which lead to microangiopathy, are of greater consequence compared to coagulopathies in children with PVHI/PVI. Both pregnancy and delivery risk factors, as well as clinical and MRI findings in children with collagenopathy are variable and different in severity. Without genetic testing, children with genetic variants leading to perinatal stroke cannot be distinguished from children without genetic variants.

Even children with mild clinical and radiological signs of stroke can have collagenopathy that leads to microangiopathy and recurrent stroke. This study suggests that all children with severe grade IVH or PVHI/PVI should be allocated for genetic testing, especially for collagenopathies. Clinical and radiological findings can not predict which children with PVHI/PVI have the genetic variants causing stroke. Genetic testing for collagenopathy is also of great value because familial cases with different clinical and radiological manifestations may have variable severities in different generations, among them pregnancy loss, fetal cerebral cortical malformations, and stroke or intracerebral hemorrhages in early adulthood.

Early diagnosis of perinatal stroke both in preterm and term born neonates contributes to the recovery process owing to neuroplasticity, however, this can be limited in very premature neonates. Therefore, a better knowledge of the risk factors and avoidance of stroke are the best options in this situation.

9. CONCLUSIONS

The study provides insight into potential antenatal and delivery risk factors for development of presumed antenatal stroke and demonstrates the impact of genetic variants on outcome in children with PVHI/PVI with implication of medical practice and health policy.

1. Pregnancy and delivery associated risk factors for PVI/PVHI and gestational age at their occurrence are similar in term born children with presumed antenatal PVI and in preterm children with PVHI, while PVI/PVHI is diagnosed at different ages. Prompt antibacterial treatment in the case of maternal bacterial infections, especially between GW 21 and 31, has a potential for prevention of presumed antenatal PVI.

   Cerebral ultrasonography is indicated for screening for severe IVH or PVHI in fetuses and term born neonates of mothers with a history of severe bacterial infection during pregnancy,
2. The profile of pregnancy and delivery associated risk factors is different in preterm neonates with antenatal onset compared to neonatal onset of severe IVH/PVHI. Bacterial infection during pregnancy is an important risk factor for development of antenatal onset severe IVH or PVHI, and delivery associated risk factors are important for neonatal onset of severe IVH/PVHI. Children with antenatal onset PVHI may have only mild symptoms after birth. In preterm neonates born to mothers with severe bacterial infection during pregnancy, cerebral ultrasonography is indicated for early detection of possible severe IVH or PVHI.
3. Children with PVHI/PVI show high prevalence of pathogenic variants in collagen genes (*COL4A1/A2* and *COL5A1*) with poor neurodevelopmental outcome. Genetic testing should be considered for all children with PVHI/PVI, while *COL4A1/A2* and *COL5A1/A2* genes should be investigated in the first place.

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11. SUMMARY IN ESTONIAN

Enneaegselt sündinud laste periventrikulaarse hemorraagilise infarkti ja ajaliselt sündinud laste periventrikulaarse venoosse infarkti riskitegurid ja tekkeaeg

Sissejuhatus

Insult võib esineda kogu elu jooksul, sealhulgas ka loote- ja vastsündinueas (Kirton and deVeber, 2013). Parem aju piltdiagnostika, sealhulgas magnet-resonantstomograafia (MRT) kättesaadavus ja paranenud teadmised arstkonna seas on perinataalse insuldi diagnoosimise sagedust tõstnud, kuid arvatakse, et insult on endiselt aladiagnoositud, eriti kergemate juhtude osas (Dunbar et al., 2020). Perinataalse insuldi kolm kõige sagedasemat vaskulaarset tüüpi on: 1) arteriaalne isheemiline insult (AII), mis esineb enamasti ajaliselt sündinud lastel sagedusega 1:1000 elussünni kohta, 2) periventrikulaarne venoosne insult (PVI), mida sageli diagnoositakse ajaliselt sündinud lastel alles imikueas (Laugesaar et al., 2007; Grunt et al., 2015; Ilves et al., 2016; Dunbar et al., 2020), 3) periventrikulaarne hemorraagiline insult (PVHI), mis esineb eelkõige enneaegselt sündinud lastel. Vaatamata paranenud intensiivravile on raske (III astme) intraventrikulaarne hemorraagia (IVH) ja PVHI jäänud olulisteks patoloogiateks enneaegsete laste hulgas, mida esineb 10–15% sügavalt enneaegsetest lastest (Ment et al., 2014, 2015; Handley et al., 2018).

Insult loote ja vastsündinu perioodil toob kaasa elukestva motoorse kahjustuse, epilepsia, kognitiivse arengu, kõne-, ja käitumishäired ning sotsiaalsed ja akadeemilised probleemid, mis on sageli kombineeritud (Mercuri, 2003; Jenks et al., 2009, 2009; Kirton and deVeber, 2013; Whittingham et al., 2014; Kuczynski et al., 2016; Lõo et al., 2018; Dunbar and Kirton, 2018; Ferriero Donna M. et al., 2019; Hawe et al., 2020; Virani et al., 2022). Akadeemilised raskused ja kognitiivne defitsiit võivad lapse kasvades süveneda, kuna suurenevad ka akadeemilised nõudmised koolis (Westmacott et al., 2009). Insult mõjutab nii lapsi kui ka nende lähedasi kogu elu jooksul ja rehabilitatsiooniks kulub suur ühiskonna ressurss. Seetõttu on oluline nii perinataalse insuldi vältimine, insuldi varane avastamine ning varane rehabilitatsioon.

Looteeas kujuneva insuldi tekkepõhjused ei ole selged, kuna selles eas on insult harva esinev ja seetõttu vähe uuritud (Özduman et al., 2004; Elchalal et al., 2005). Ei ole isegi teada, millal täpselt looteeas PVI tekib, kuid oletatakse, et see tekib sarnaselt enneaegselt sündinud laste insuldiga enne 34. rasedusnädalat (RN.) (Kirton et al., 2010; Ilves et al., 2016; Dunbar ja Kirton, 2018). Looteeas tekkinud insuldi korral ei esine sünnijärgselt insuldile iseloomulikke sümptome ja neuroloogilised sümptomid kujunevad alles imikueas, mistõttu teostatakse aju MRT uuring, kus ilmestub insuldile iseloomulik leid – hilise diagnoosiga PVI (Ilves et al., 2016). Insuldi korral, sealhulgas ka looteeas tekkinud insuldi puhul, on aga oluline alustada rehabilitatsiooniga võimalikult varakult.

Enneaegsetel lastel on raske IVH/PVHI etioloogia multifaktoriaalne (Ment et al., 2015). Enamus seniseid etioloogia ja patogeneesi alaseid uurimistöid on keskendunud sünnitus- ja intensiivravi aegsetele riskiteguritele, mis mõjutavad aju verevoolu sügavalt enneaegsetel vastsündinutel, kellel esineb ebaküpsete veresoontega germinaalmaatriks ("Volpe's Neurology of the Newborn – 6th Edition," n.d.). Ema transport suurematesse keskustesse, sünnieelse steroidi ja postnataalse surfaktandi kasutamine, täiustunud elustamisvõtted ja intensiivravi pole siiski toonud kaasa raske IVH ja PVHI haigestumuse olulist vähenemist viimasel 10-15 aastal (Stoll et al., 2010; Ment et al., 2015; Inder et al., 2017), kuna elama jäävad järjest enneaegsemad lapsed. Mõnel enneaegsel lapsel on raske IVH/PVHI nähtav aju ultraheliuuringul juba esimestel elutundidel ja kahjustust võib pidada looteeas kujunenuks (de Vries et al., 1998; Harteman et al., 2012). Parem arusaamine PVHI teket mõjutavatest üsasisestest riskiteguritest võib aidata vältida raske IVH/PVHI kujunemist enneaegselt sündinud lastel.

Geneetiline eelsoodumus võib mõjutada looteea insuldi ja raske hemorraagia teket enneaegsetel lastel ja see võib osal lastest olla juhtivaks teguriks PVHI tekkes (Ment et al., 2014, 2015). Ühes varasemas töös leiti, et trombofiilia risk ei olnud kõrgem neonataalse tekke ja hilise diagnoosiga AISiga lastel ega hilise diagnoosiga looteea PVIga lastel (Curtis et al., 2017). Loodete hulgas, kellel olid ajus tekkinud multifokaalsed hemorraagiad ja/või isheemilised kahjustuskolded, leiti tihti kollagenopaatiaid (Matsumoto et al., 2015; Maurice et al., 2021). Varasemalt ei ole PVHI/PVI leiuga enneaegseid ja ajalisi lapsi kollagenopaatiate suhtes geneetiliselt uuritud, kuigi kollagenopaatiad on seotud mikroangiopaatiaga ja võivad olla hemorraagia tekke põhjuseks (Knaap et al., 2006).

Uuringu eesmärgid ja hüpoteesid

Spetsiifilised eesmärgid ja hüpoteesid olid:

1. Võrrelda ajalisi ja peaaegu ajalisi lapsi (sündinud ≥36 RN.) hilise PVI diagnoosiga, enneaegselt sündinud lapsi (sündinud <36 RN.) PVHIga ja järjestikuselt kogutud kontrollrühma lapsi (sündinud ≥36 RN.), et kindlaks teha rasedus- ja sünnitusaegsete riskitegurite levimus ja raseduse suurus tegurite esinemise ajal (Uuring I).

   Hüpoteesiks oli, et rasedus- ja sünnitusaegsed riskitegurid ning nende esinemise aeg on sarnased nii PVHI kui ka PVIga lastel, kuigi PVHI/PVI diagnoositakse erinevatel aegadel (Uuring I).

2. Võrrelda rasedus- ja sünnitusaegseid riskitegurid raske IVH/PVHI diagnoosiga enneaegselt sündinud lastel, kellel oli insult nähtav aju ultraheliuuringul esimese 12 elutunni jooksul ja seega tekkinud looteeas, ning nendel enneaegsetel lastel, kellel kahjustus kujunes hiljem vastsündinuperioodis.

   Hüpoteesiks oli, et rasedus- ja sünnitusaegsete riskitegurite profiilid on looteea tekkega IVH/PVHI-ga ja neonataalse tekkega IVH/PVHIga enneaegsetel lastel erinevad (Uuring II).

3. Uurida varasemalt insuldi tekkega seotud patogeensete geenivariantide levimust PVHI/PVIga lastel.

Hüpoteesiks oli, et PVHI/PVIga lastel on kõrge kollagenopaatiate levimus sarnane porentsefaaliaga loodete ja laste varasematel uuringul leitud levimusega (Uuring III).

Uuritavad ja meetodid

Uuringusse kaasati ajaliselt sündinud looteea PVIga lapsed, hilise diagnoosiga ilmselt looteea PVIga lapsed ja enneaegselt sündinud PVHIga lapsed Eesti laste insuldi andmekogust (ELIA). Uuringusse kaasati PVIga lapsed, kes sündisid aastatel 1994 kuni 2019 ja olid ravil Tartu Ülikooli Kliinikumis. ELIAsse on kaasatud ajaliselt sündinud perinataalse insuldiga lapsed, kellel on tehtud insulti kinnitav kompuutertomograafia või MRT uuring ja kelle puhul insult on klassifitseeritud vaskulaarse geneesi järgi vähemalt kahe neuroradioloogi ja lasteneuroloogide poolt. Enneaegsed raske vatsakesesisese hemorraagia või PVHIga lapsed leiti Tartu Ülikooli Kliinikumi andmekogust, sealhulgas laste intensiivravi osakonna andmebaasist ja ka haigla digitaalsest infosüsteemist. PVHI enneaegsetel lastel diagnoositi aju ultraheli uuringu alusel. Diagnoosi kinnitas MRT uuring, mis teostati postmenstruaalvanuses 40. nädalat või hiljem, või lahang. Radioloogiliselt ja kliiniliselt kinnitatud juhud lisati ELIAsse.

PVHI/PVI on aju ultraheliuuringul defineeritud kui aju parenhüümi ehhogeensuse tõus periventrikulaarses piirkonnas. Kahjustus võib olla lehviku või keraja kujuga ning võib asetseda eraldi või ühenduses aju külgvatsakesega ning areneb hiljem kas väikeste periventrikulaarsete tsüstidega piirkonnaks või suureks porentsefaalseks tsüstiks koos samapoolse külgvatsakese laienemisega (de Vries et al., 2001; Dudink et al., 2008; Elchalal et al., 2005; Rademaker et al., 1994).

MRT-uuringul on PVHI/PVI defineeritud valdavalt ühepoolse medullaarveeni territooriumi infarktina periventrikulaarses piirkonnas koos aju külgvatsakese porentsefaalse laienemisega ja/või periventrikulaarse glioosiga ja/või väikeste periventrikulaarsete tsüstidega või suurema porentsefaalse tsüstiga. Hemosideriini ladestumine võib olla nähtav germinaalmaatriksi piirkonnas, aju külgvatsakeses ja/või periventrikulaarses piirkonnas (Takanashi et al., 2003; Elchalal et al., 2005; Kirton et al., 2008; Kirton and Wei, 2010; Kirton et al., 2010).

ELIA andmebaasist väljaarvamise kriteeriumiteks olid 1) puuduv aju MRT või kompuutertomograafiline uuring või lahang; 2) muud kesknärvisüsteemi haaravad haigused nagu näiteks a) raske sünniasfüksia koos veelahkme piirkonna infarktide või periventrikulaarse leukomalaatsiaga; b) infektsioossed haigused, kaasa arvatud meningoentsefaliit koos ajuinfarktidega; c) metaboolsed ja mitokondriaalsed haigused; d) tuumorid; e) teised kaasasündinud anomaaliad või kortikaalse arengu häired.

I ja II uuringu kontrollgrupi emad ja lapsed leiti prospektiivsest rasedate uuringust „Happy Pregnancy Study“ (juhtivuurija Prof. Maris Laan, http://www.happypregnancy.ut.ee/en). Uuringusse kaasati 2/3 kõigist rasedatest, kes olid rasedusaegsel jälgimisel Tartu Ülikooli Kliinikumi naistekliinikus aastatel 2013-2015 (n=2359).

I uuringusse kaasati emad ja nende PVIga lapsed (≥36 RN., n=43), emad ja enneaegselt sündinud PVHIga lapsed (<36 RN., n=86) ja kontrollrühma emad ja nende ajaliselt sündinud lapsed (n=2168).

II uuringusse kaasati emad ja nende enneaegselt sündinud PVHIga lapsed (<35 RN., n=120) ning kontrollrühma emad ja nende enneaegsed lapsed (<35 RN., n=50). Uuringurühma lapsed jagati looteea algusega IVH/PVHI (muutused nähtavad UH-uuringul <12h, n=27), neonataalse algusega IVH/ PVHI (muutused tekkinud pärast esmast normis ultraheliuuringut, n=70) ja ebaselge algusega IVH/PVHI (esmane ultraheliuuring teostatud >12h, n=23) gruppidesse.

III uuringusse kaasati ajalised looteea PVIga lapsed (n=6), hilise diagnoosiga, ilmselt looteea PVIga lapsed (n=40) ja enneaegselt sündinud IVH/PVHIga lapsed (n=39).

Kliiniline informatsioon raseduse, sünnituse ja vastsündinuperioodi kulu kohta saadi emade ja laste andmetest paberdokumentides ja digitaalsetes arhiivides. Andmeid koguti kolmest regionaalsest haiglast, neljast keskhaiglast ja kaheksast maakonnahaiglast, kus uuringuga seotud laste emad olid rasedusega arvel või sünnitasid.

Koguti andmed reproduktiivanamneesi, vanemate elustiili, käesoleva raseduse ja sünnituse kulu ning vastsündinuperioodi kohta. Emade rasedusaegse antibakteriaalse ravi kohta saadi andmed rasedus- ja sünnituslugudest ning kontrollgrupil retseptikeskusest. Infektsiooni diagnoos klassifitseeriti vastavalt WHO rahvusvahelise haiguste klassifikatsiooni 10. versioonile (“ICD-10 Version:2019,” n.d.).

Geneetilisteks uuringuteks analüüsiti verest eraldatud leukotsüütides olevat DNA-d kasutades next-generation sequencing (NGS) tehnikat (23 juhul) ja kogu eksoomi sekveneerimise analüüsi (60 juhul). Kahel perekondlikul juhul teostati üksikgeenianalüüs.

Uuringus kasutati andmeid Eesti Tervishoiu Pildipangast ja MRT uuringud tehti 3 teslase seadmega standardprotokolli järgi laste vanuses 7–18 aastat, kui uuringut polnud varem kliinilisel eesmärgil teostatud. MRT uuringute läbiviimiseks teadusuuringu raames anesteesiat ei kasutatud. Laste neurokognitiivse arengu hindamist vanuses 7–18 aastat viisid läbi lasteneuroloogid, psühholoogid ja füsioterapeudid.

Tulemused

Esimeses uuringus ilmnes, et hilise diagnoosiga ilmselt antenataalse PVIga laste emadel oli oluliselt rohkem rasedusaegseid bakteriaalseid infektsioone võrreldes kontrollgrupiga ($p<0,001$), sealhulgas kuse-suguteede infektsioone ($p=0,001$) ja

spetsiifilisemalt püelonefriiti (p<0,001). Ema bakteriaalne infektsioon oli sage nii hilise diagnoosiga looteea PVIga laste rühmas (20/43; 47%) kui ka enneaegselt sündinud PVHIga laste rühmas (42/86; 49%) (p=0,72).

Kuse- ja suguteede infektsioonide levimus oli sage nii hilise diagnoosiga looteea PVIga (10/43; 23%) kui ka enneaegselt sündinud PVHIga (15/86; 17%) laste emade hulgas (OR, 1.4 [95% CI, 0,6–3,5], p=0,43). Püelonefriit moodustas sarnaselt suurima proportsiooni kõikidest infektsioonidest hilise diagnoosiga looteea PVIga rühmas ja PVHIga rühmas, vastavalt 10/20 (50%) versus 15/42 (36%), (OR, 1,8 [95% CI, 0,6–5,3], p=0,28). Erinevalt uuringurühmadest moodustas püelonefriit kontrollrühmas ainult väikese osa kõikidest infektsioonidest: 15/437 (3.4%) kontrollrühmas ja 10/20 (50%) hilise diagnoosiga looteea PVIga rühmas (OR, 28 [95% CI, 10–78], p<0,001). Ema püelonefriiti oli hilise diagnoosiga looteea PVIga lastel (10/43; 23%) oluliselt rohkem kui kontrollgrupi lastel (15/2168; 0.7%) (OR, 43 [95% CI, 16–112], p<0.001).

Ema infektsiooni tekke ajas hilise diagnoosiga looteea PVIga rühma ja PVHIga rühma vahel erinevusi ei leitud (vastavalt mediaan rasedusnädalad (kvartiilhaare) (26,5 [21–31] versus 23,5 [21,5–27]; p=0.24). Kontrollrühma emadel leiti infektsioone oluliselt varasemas rasedusjärgus (OR, 16,4 [95%CI, 12,4–26,1], p=0,001) võrreldes hilise diagnoosiga looteea PVI rühmaga.

Teises uuringus ilmnes, et looteea algusega PVHIga enneaegselt sündinud vastsündinute emadel esines sagedamini bakteriaalseid infektsioone võrreldes neonataalse algusega PVHIga rühmaga (aOR, 5.7 [95% CI, 2,1–16], p=0,0008). Kuse- ja suguteede infektsioonide, sealhulgas püelonefriidi, levimus oli suurem looteeas tekkinud PVHI-ga laste emade hulgas võrreldes neonataalse algusega PVHI-ga laste emade hulgas (p=0,005), sarnane seos esines ka teiste infektsioonide korral (p=0,006). Looteea algusega PVH-ga rühmas oli rohkem ka kliiniliselt diagnoositud (p=0,025) ja histoloogiliselt kinnitatud (p=0,025) koorioamnioniite võrreldes neonataalse algusega PVHIga rühmaga.

Samal ajal oli looteea ja ebaselge algusega PVHI-ga vastsündinutel vähem loote või neonataalse stressi ning komplikatsioonidega seotud tegureid, sealhulgas erakorralist keisrilõiget, surfaktanti ja kardio-või vasoaktiivsete ravimite vajadust ning juhitavat ventilatsiooni üle 24 tunni võrreldes neonataalse algusega PVHI grupiga.

Kolmandas uuringus leiti patogeensed ja tõenäoliselt patogeensed variandid geenides, mis on seotud kollagenopaatiatega ja koagulopaatiatega, 11/85 (12,9%) PVHI või PVIga patsientidel. Viiel nendest lastest avastati muutused teadusuuringu käigus; geneetilist haigust ei olnud uuritavatel varem kahtlustatud. Patogeensete või tõenäoliselt patogeensete geenivariantidega laste hulgas leiti 7/11 (63%) muutused *COL4A1/A2* ja *COL5A1* geenides. Kuuel juhul üheteistkümnest muutustega lastest leiti geenivariandid, mida kirjeldati esmakordselt selles töös.

Lastel, kellel leiti patogeenne geenivariant kollageeni geenis, oli hilisemas lapseeas rohkem mõõdukat kuni rasket motoorset defitsiiti (85,7%) võrreldes lastega, kellel geneetilisi muutusi ei leitud (20,7%), (OR, 233 [95%CI, 2,8 – 531], p=0,0013). Samuti oli muutunud kollageeni geeniga lastel rohkem epi-

lepsiat võrreldes lastega, kellel muutusi ei leitud (vastavalt 57.1% versus 15,5%, OR,7,3 [95%CI, 1,3 – 41], p=0,025).

Kuigi neljal seitsmest kollagenopaatiaga lapsel esinesid suured muutused koos multifokaalsete kahepoolsete insultidega ja valgeaine mahu vähenemisega aju MRT-uuringul, esinesid kolmel kollagenopaatiaga lapsel ainult ühepoolsed muutused (p=0,048). Seega MRT-uuringuga ei saa kollagenopaatiaga PVHI/PVIga lapsi eristada geenimuutusteta lastest, mistõttu on vaja teha geneetilised uuringud.

Kõik uuringud olid mõjutatud sarnastest uuritavate rühmade piirangutest.

Rasedus- ja sünnitusaegseid riskitegureid koguti retrospektiivselt erinevatest haiglatest kasutades standardiseeritud definitsioone. Uuring võis alahinnata infektsioonide hulka PVHI/PVI grupis puuduliku dokumentatsiooni tõttu. Samas kontrollgrupi emade kohta oli andmeid kõigi väljakirjutatud antibiootikumide kohta retseptikeskuses olemas, kusjuures neil oli ikka vähem infektsioone. Uuringuperiood oli pikk, mille jooksul muutus enneaegsete laste ravistrateegia, seetõttu tulemusi korrigeeriti sünniaasta suhtes. Geneetilist testimist ei tehtud kõigile uuringugrupi lastele. Kuna geenivariante uuriti vaid üksnes vähestel PVHIga lastel, kes surid, siis võis geenivariantide hulk olla alahinnatud.

Järeldused

Uurimus käsitleb sünnieelsete ja sünnitusaegsete riskitegurite ning geneetiliste variantide mõju PVHI/PVI geneesis koos võimalike mõjutustega kliinilisele praktikale ja tervisepoliitikale.

1. Raseduse ja sünnitusega seotud riskitegurid ning nende esinemise aeg on sarnased ajaliselt sündinud hilise diagnoosiga looteea PVIga lastel ja enneaegselt sündinud PVHIga lastel, kuigi insuldi diagnoosimise aeg oli erinev. Ema bakteriaalseid infektsioone, eriti 21. ja 31. rasedusnädala vahel, tuleks viivitamatult ravida, et vältida võimalikku PVI teket looteeas. Looted ja ajaliselt sündinud vastsündinud, kelle emadel on raseduse ajal olnud infektsioon, vajavad aju ultraheliuuringut, et avastada varakult võimalik raske IVH/PVHI.
2. Raseduse ja sünnitusega seotud riskitegurid neonataalse ja antenataalse tekkega raske IVH/PVHI korral enneaegselt sündinud lastel on erinevad. Bakteriaalne infektsioon raseduse ajal on oluline riskitegur antenataalse raske IVH/PVHI kujunemisel ning sünnitusega ja intensiivraviga seotud riskitegurid on olulised neonataalse PVHI kujunemisel. Antenataalse PVHIga lastel võivad olla sünnijärgselt ainult kerged sümptomid. Enneaegselt sündinud vastsündinud, kelle emadel on raseduse ajal olnud bakteriaalne infektsioon, vajavad varast aju ultraheliuuringut, et avastada varakult võimalik raske IVH/PVHI.
3. Kõrge patogeensete variantide levimus kollageeni geenides (COL4A1/A2 ja COL5A1) esineb PVHI/PVIga laste hulgas, kellel on ka halb neuroarenguline hilistulemus. PVHI/PVIga lastel tuleb kaaluda geneetiliste testide

tegemist ja erilist tähelepanu peab pöörama muutustele COL4A1/A2 ja COL5A1 geenides.

Insuldiga lastel on suur risk eluaegseks püsivaks kahjustuseks. Insulti on raske ravida ja ka parim rehabilitatsioon ainult kergendab sümptome, kuid ei ravi lapsi terveks. Et vältida eluaegset puuet, parandada nende laste ja perede elukvaliteeti ning vähendada sotsiaalset ja majanduslikku koormat ühiskonnale, on insuldi vältimine parim vahend. Rasedusaegse infektsiooni viivitamatu ravi ja geeniuuringud pakuvad selleks võimalust. Geeniuuringud võimaldavad ennustada halba prognoosi, epilepsia kujunemist ning insuldi võimalikku kordusriski perele.

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