DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
172

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
172

RAEL LAUGESAAR

Paediatric stroke in Estonia: epidemiology and risk factors

TARTU UNIVERSITY
PRESS

Department of Paediatrics, Faculty of Medicine, University of Tartu, Tartu, Estonia

Dissertation is accepted for the commencement of the degree of Doctor of Medical Sciences on April 21, 2010 by the Council of the Faculty of Medicine, University of Tartu, Tartu, Estonia.

Supervisors: Professor Emeritus Tiina Talvik, MD, DMSc, Department of Paediatrics, Faculty of Medicine, University of Tartu, Tartu, Estonia

Senior Research Fellow Anneli Kolk, MD, PhD, Department of Paediatrics, Faculty of Medicine, University of Tartu, Tartu, Estonia

Reviewers: Associate Professor Oivi Uibo, MD, DMSc, Department of Paediatrics, University of Tartu, Tartu, Estonia

Senior Research Fellow Janika Kõrv, MD, DMSc, Department of Neurology, University of Tartu, Tartu, Estonia

Opponent: Professor of Paediatric Neurology Fenella Kirkham, MA, MB, BCh, MRCP, FRCP, UCL, Neurosciences Unit, Institute of Child Health (University College London), London, United Kingdom

Commencement: June 18, 2010

Publication of this dissertation was supported by the University of Tartu and by the European Union through the European Social Fund.

ISSN 1024–395X
ISBN 978–9949–19–376–9 (trükis)
ISBN 978–9949–19–377–6 (PDF)

Tartu Ülikooli Kirjastus
www.tyk.ee
Tellimus nr. 240

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I Laugesaar R., Kolk A., Tomberg T., Metsvaht T., Lintrop M., Varendi H., Talvik T. Acutely and retrospectively diagnosed perinatal stroke: A population-based study. Stroke 2007; 38: 2234–2240.

II Laugesaar R., Kolk A., Uustalu Ü., Ilves P., Tomberg T., Talvik I., Köbas K., Sander V., Talvik T. Epidemiology of childhood stroke in Estonia. Pediatric Neurology 2010; 42: 93–100.

III Laugesaar R., Kahre T., Kolk A., Uustalu Ü., Kool P., Talvik T. Factor V Leiden and prothrombin 20210G>A mutation and paediatric ischemic stroke: a case-control study and two meta-analyses. Acta Paediatrica 2010; in press. Published Online: March 2, 2010.

Applicant's contribution to these publications:

Paper I: Study design, finding patients, data collection, data analysis and writing the paper.
Paper II: Study design, finding patients, data collection, data analysis and writing the paper.
Paper III: Study design, finding patients, partial performing of gene analysis, data analysis and writing the paper.

ABBREVIATIONS

AIS	Arterial ischemic stroke
CI	Confidence interval
CT	Computed tomography
EEG	Electroencephalogram
FVL	Factor V Leiden
IPH	Intraparenchymal haemorrhage
MTHFR	Methylenetetrahydropholate reductase
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
OR	Odds ratio
PT	Prothrombin
SVT	Sinovenous thrombosis
SAH	Subarachnoid haemorrhage
TIA	Transient ischemic attack
WHO	World Health Organisation

1. INTRODUCTION

Stroke is an increasingly recognised cause of childhood mortality and long-term neurological morbidity. In the United States, stroke is among the top 10 causes of death among children (*Mallick et al., 2010a*). Paediatric stroke has a large and sustained economic burden, with the five-year health care cost being 15 times that of age-matched children who have not suffered from a stroke (*Gardner et al., 2010*). Approximately 60 percent of children who have suffered from a stroke have residual impairments that influence their daily life (*Ganesan et al., 2000*). The World Health Organisation (WHO) defines a stroke as "rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than vascular origin" (*Hatano, 1976*). Thrombosis or embolus may occur in either the arterial or venous systems. Depending on the system involved, childhood stroke is divided into arterial and venous (sinovenous) stroke. Arterial stroke can be either ischemic (arterial ischemic stroke, AIS) or haemorrhagic (intraparenchymal haemorrhage, IPH, or subarachnoid haemorrhage, SAH). In case of venous stroke (sinovenous thrombosis, SVT), the primary event is ischemic (thrombus or embolus) but the parenchymal damage may either be haemorrhagic infarct or pure ischemic lesion. AIS accounts for 70 percent of paediatric stroke, with haemorrhagic stroke representing 20 percent and SVT constituting 10 percent (*Govaert et al., 2009a*).

Stroke occurs in children of all ages. As stroke in neonates differ from stroke in older infants and children, paediatric stroke is divided into perinatal and childhood stroke (Figure 1). The time interval of a perinatal stroke is from 20 gestation weeks through the 28th postnatal day (*Raju et al., 2007*), while a childhood stroke occurs between one month and 18 years of age (*Amlie-Lefond et al. 2008*). The term 'paediatric stroke' encompasses both perinatal and childhood strokes. Perinatal stroke is responsible for 35–46 percent of all cases of paediatric ischemic stroke (*Steinlin et al., 2005; Fullerton et al., 2007a*).

Paediatric stroke in Estonia is yet to be investigated and the incidence of stroke among children in this country is unknown. At the same time, adult stroke epidemiology has been studied in depth in Tartu, Estonia. The third adult stroke registry was conducted from 2001 to 2003 by Vibo et al. (2007), who found the crude incidence rate of first-ever stroke 223 per 100,000 person-years.

There is a need for epidemiological studies in every country in order to obtain evidence-based data about the profile of the disease, which can provide important data for planning medical and social care. The present study was designed to investigate the incidence rate of paediatric stroke in Estonia and to evaluate the risk factors of paediatric stroke.

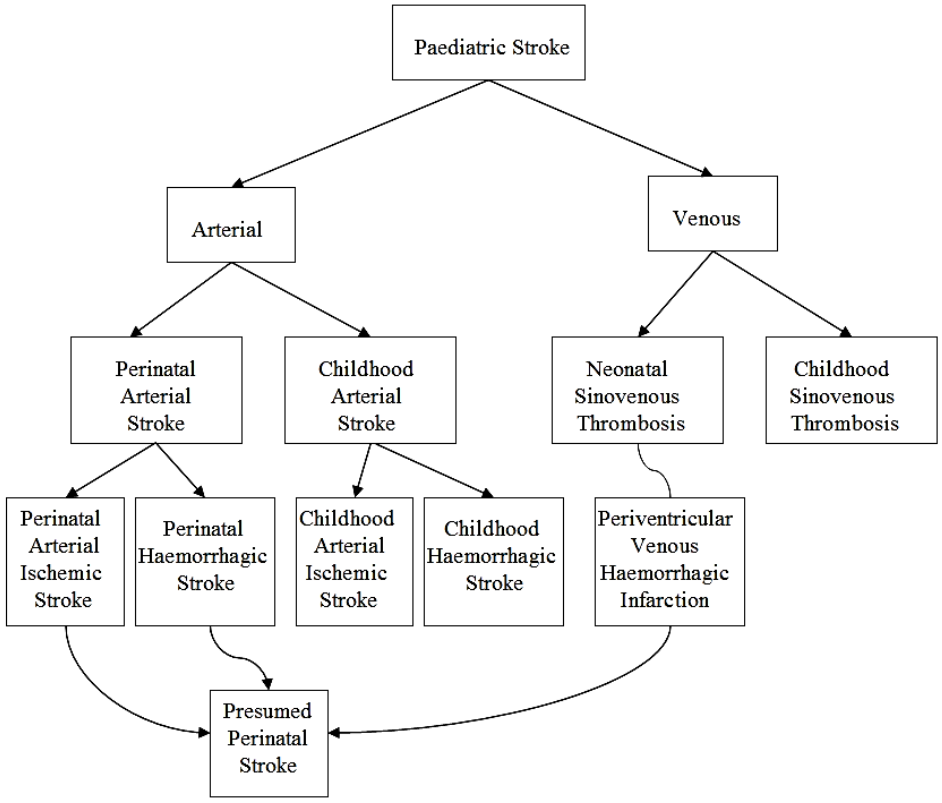

Figure 1. Scheme for classification of paediatric stroke

2. LITERATURE REVIEW

2.1. Perinatal arterial stroke

2.1.1. Definition and classification

According to the accepted definition, perinatal stroke is "a vascular event causing focal interruption of blood supply, occurring between 20 weeks of foetal life through 28th postnatal day, and confirmed by neuroimaging or neuropathology studies" (*Raju et al., 2007; Lynch, 2009*). Different from the WHO definition, the diagnosis of a perinatal stroke is based on neuroradiological confirmation of a vascular event rather than the typical clinical presentation of a stroke, which is often absent in the case of a perinatal stroke.

Perinatal stroke is classified according to two major subtypes: ischemic (AIS and SVT) and haemorrhagic perinatal stroke (*Lynch, 2009*). It is often difficult to distinguish between these vascular events of perinatal stroke, and differentiating the haemorrhagic conversion of ischemic lesions from a primary brain haemorrhage can be impossible (*Raju et al., 2007*). Venous infarcts are often haemorrhagic to begin with; ischemic infarcts may become haemorrhagic after reperfusion and one patient may have many different vascular pathologies (*Raju et al., 2007*).

The timing of the vascular event that leads to a perinatal stroke is almost always unknown. Therefore, classification of perinatal strokes is based on the gestational or postnatal age at diagnosis. A **foetal stroke** is diagnosed before birth by using foetal imaging methods or in stillbirths on the basis of neuropathologic examination. A **neonatal stroke** is diagnosed after birth or before the 28th postnatal day (including in preterm infants). A **presumed perinatal stroke** is diagnosed in infants aged more than 28 days in whom it is presumed (but not certain) that the vascular event occurred sometime between the 20th week of foetal life and the 28th postnatal day (*Raju et al., 2007*).

It has been suggested that cases of IPH and SAH should be included in perinatal haemorrhagic stroke studies (*Govaert et al., 2009a*), in which SAH constitute only a minority of the perinatal haemorrhagic stroke cases (*Armstrong-Wells et al., 2009*). Perinatal intraventricular haemorrhage is believed to be distinct from perinatal IPH and SAH (*Armstrong-Wells et al., 2009*).

Perinatal stroke most often occurs in term or near-term infants but also sometimes in preterm infants (*Benders et al., 2008; Golomb et al., 2008a*). Accordingly, most reports on perinatal stroke have focused on term or near-term patients (*Raju et al., 2007*).

Since neuroradiological investigations are performed in the acute phase, both foetal and neonatal stroke can be classified as either ischemic or haemorrhagic in origin. The distinction between the ischemic or haemorrhagic origin of a

presumed perinatal stroke is often difficult because the neuroradiological investigations are delayed due to the absence or subtlety of neurological symptoms in the neonatal period (*Armstrong-Wells et al., 2009*).

2.1.2. Epidemiology of perinatal stroke

The estimated incidence rates of perinatal stroke vary widely, depending on the methodology of the study. Previously published perinatal stroke studies have typically been restricted only to AIS and have included only children with neonatal stroke (*Perlman et al., 1994; Estan and Hope, 1997; Govaert et al., 2000; Schulzke et al., 2005; Lynch et al., 2002*). The incidence rate of neonatal AIS in these studies ranges from 17.8 to 43.4 per 100,000 live births (Table 1). Two published population-based studies that also involved cases with presumed perinatal stroke showed the incidence rate of perinatal stroke to be 17.0 and 20.0 per 100,000 live births, respectively (*Wu et al., 2004; Lee et al., 2005*; see Table 1). It is difficult to explain why the incidence rate is not higher in studies that include patients with both neonatal and presumed perinatal strokes (Table 1). In these studies, children with neonatal stroke constituted 33–58 percent of the total perinatal stroke cohort. According to previous results on the incidence of neonatal stroke, this proportion should have yielded an incidence rate of perinatal stroke of between 30 and 129 per 100,000 live births (Table 1).

The population-based incidence rate of neonatal stroke among premature infants has not been studied. Hospital-based studies have reported that neonatal AIS develops in 0.7–1.0 percent of preterm infants aged ≤34 gestational weeks who are admitted to neonatal intensive care units (*de Vries et al., 1997; Benders et al., 2008*).

Three studies have estimated the incidence of perinatal haemorrhagic stroke (*Lynch et al, 2002; Armstrong-Wells et al., 2009; Sachs et al., 1987*). Sachs et al. reported an incidence rate of almost 10 times that observed by Lynch et al. and Armstrong-Wells et al. (Table 1). In contrast to the findings of Lynch et al. and Armstrong-Wells et al., 11 out of 12 of Sachs et al. cases had SAH.

Table 1. Previous population-based studies on perinatal arterial stroke

Reference	Country	No of Patients	Gestational Age	IR per 100,000 Live Births
Neonatal AIS				
Perlman, 1994	USA	8	term	28.6
Estan, 1997	UK	12	>31 GW	24.7
Govaert, 2000	Belgium	13	>30 GW	35.0
Schulzke, 2005	Switzerland	9[a]	mostly term	43.4
NHDS,[b] 1980–1998	USA	unknown	mostly term	17.8
Armstrong-Wells, 2009	USA	93	>28 GW	29.0
Neonatal haemorrhagic stroke				
NHDS,[b] 1980–1998	USA	unknown	mostly term	6.7
Armstrong–Wells, 2009	USA	20	>28 GW	6.2
Sachs, 1987	USA	12	term	52.0
Both neonatal and presumed perinatal stroke included				
Wu, 2004	USA	38	term	17.0
Lee, 2005	USA	40	>30 GW	20.0

IR-incidence rate; GW – gestational weeks
[a]Includes one patient with presumed perinatal stroke, [b]National Hospital Discharge Survey, reported by Lynch et al. (2002)

All previously published studies have been retrospective. When earlier studies were hospital-based, the latter studies were population-based (*Lynch et al., 2002; Wu et al., 2004; Lee et al., 2005*). However, the search criteria have varied among studies. Some have been based on clinical presentation (*Perlman et al., 1994; Estan and Hope, 1997; Wu et al., 2004; Armstrong-Wells et al., 2009*), some have been based on hospital discharge codes (*Lynch et al., 2002*) and others have been based on neuroimaging reports (*Govaert et al., 2000; Schulzke et al., 2005; Lee et al., 2005*).

Most studies have reported male predominance (54–75 percent) among perinatal stroke patients (*Perlman et al., 1994; Golomb et al., 2004; Chabrier et al., 2010; Herak et al., 2009*).

The mortality rate following perinatal AIS is low, and death is often associated with comorbidities such as sepsis, meningitis, severe prothrombotic disorder, complex congenital heart disease and extreme prematurity (*Lee et al., 2005; Golomb 2009*).

2.1.3. Clinical presentation of perinatal stroke

Some children with perinatal stroke are symptomatic during the neonatal period (neonatal stroke), while other children have subtle neurological symptoms after birth (or none at all) and only come to the physicians' attention months later because of hemiparesis or seizures (presumed perinatal stroke) (*Golomb et al.,*

2001; Raju et al., 2007). The proportion of patients with neonatal stroke varies from 33 to 58 percent in population-based studies (*Wu et al., 2004; Lee et al., 2005*).

Term newborns with AIS most often experience seizures during the neonatal period. Seizures occur in 59–92 percent of newborns with AIS, with focal seizures occurring more often (63–100 percent) than generalized seizures (up to 37 percent) (*Fujimoto et al., 1992; Jan and Camfield, 1998; Sreenan et al., 2000; Wu et al., 2004; Golomb et al., 2008b; Chabrier et al., 2010*). Compared to hypoxic-ischemic encephalopathy, seizures in stroke patients have more delayed onset (81 percent after 12 hours of life) and focal seizures occur more often (*Rafay et al., 2009b*). Newborns may also present with apnoea (*Fujimoto et al., 1992; Jan and Camfield, 1998; Sreenan et al., 2000; Golomb et al., 2008b*) or symptoms of encephalopathy (*Sreenan et al., 2000; Ramaswamy et al., 2004; Cowan et al., 2003*). Hemiplegia or hemiparesis is diagnosed only in the minority of the newborns with AIS (*Chabrier et al., 2010*). Preterm children with neonatal AIS most commonly present with respiratory difficulties or apnoea (83 percent), seizures (25–30 percent), poor feeding (26 percent) and abnormal tone (22 percent) (*Golomb et al., 2008; Benders et al., 2008*).

The signs and symptoms of haemorrhagic stroke can be subtle and non-specific in neonates, especially if the haemorrhages are small. Some haemorrhages in neonates may even remain unrecognised (*Roach et al., 2008*). Most frequently, newborns with haemorrhagic stroke present with seizures and signs of increased intracranial pressure (*Sandberg et al., 2001; Armstrong-Wells et al., 2009*).

Infants with presumed perinatal stroke present with hemiparesis (75–100 percent), delayed developmental milestones (eight percent), and/or seizures (9–36 percent) months after birth (*Wu et al., 2004; Golomb et al., 2001; Kirton et al., 2008; Golomb et al., 2008b*). A study by Kirton et al. (2008) revealed that the median age of noticing symptoms (parental concern) was 5.0 ± 2.1 months (range: 1.3–77 months) and there were substantial delays between parental or physician concern (9.9 ± 3.6 months) and final diagnosis (12.6 ± 3.1 months).

2.1.4. Risk factors of perinatal stroke

The aetiology of neonatal stroke is poorly understood and is different from that of adult and childhood stroke (*Bernard and Goldenberg, 2008*). Most studies of perinatal stroke have been descriptive, reporting several maternal and neonatal disorders in infants with perinatal stroke. Lynch (2009) classified the aetiologies of perinatal stroke as follows: (1) maternal disorders, (2) placental disorders, (3) blood, homocysteine and lipid disorders, (4) cardiac disorders, (5) infectious disorders and (6) miscellaneous disorders. Lynch (2009) also emphasised the likelihood that perinatal factors play a role in perinatal stroke, as the recurrent-stroke rate is extremely low.

Few case-control studies have been published with contradictory results, mainly because of the small study groups and varied inclusion criteria (*Lynch, 2009*). Five case-control studies have estimated the association between maternal and perinatal factors and AIS in full-term (*Estan and Hope, 1997; Wu et al., 2004; Lee et al., 2005; Chabrier et al., 2010*) and premature neonates (*Benders et al., 2007*). In the univariate analysis, the following results were associated with perinatal AIS: primiparity (2/4)*, history of foetal loss (1/3), intrauterine growth restriction (1/3), twin-gestation (1/1), twin-to-twin transfusion syndrome (1/1), preeclampsia (1/3), oligohydramnion (1/1), reduced foetal movement (2/2), chorionamnionitis (1/1), prolonged rupture of membranes (1/2), premature rupture of membranes (1/2), prolonged second stage of delivery (1/1), foetal heart rate abnormalities (2/3), cord pathology (1/2), vacuum delivery (1/2), emergency caesarean section (3/4), Apgar score <7 at one minute (2/2) and at five minutes (2/4), resuscitation at birth (4/4), and hypoglycaemia in premature infants (1/1). Multivariate risk factor analyses revealed that the independent risk factors for perinatal AIS were history of infertility (1), intrauterine growth restriction (1), preeclampsia (2), chorionamnionitis (1), twin-to-twin transfusion syndrome (1), prolonged rupture of membranes (1), foetal heart rate abnormalities (1) and hypoglycaemia in premature infants (1). A study by Simchen et al. (2009) assessed the association between maternal prothrombotic disorders and perinatal AIS. That study found that maternal factor V Leiden (FVL), prothrombin (PT) 20210G>A mutation, methylenetetrahydropholate reductase (MTHFR) gene 677T polymorphism and maternal antiphospholipid antibodies were associated with perinatal AIS.

Seven studies have investigated the association between prothrombotic factors and perinatal stroke (mostly neonatal AIS) (*Hagstrom et al., 1998; Günther et al., 2000; Aronis et al., 2002; Hogeveen et al., 2002; Miller et al., 2006; Simchen et al., 2009; Herak et al., 2009*). These studies found that the factors associated with perinatal stroke included: FVL (4/6)*, PT 20210G>A mutation (1/5), protein C deficiency (1/3), lipoprotein (a) >30mg/dl (1/2), hyperhomocysteinemia (1/1) and antiphospholipid antibodies (1/1).

Few studies have investigated the risk factors for perinatal haemorrhagic stroke. Armstrong-Wells et al. (2009) mainly studied cases of neonatal IPH and found that an underlying aetiology was present in 25 percent of them [thrombocytopenia (20 percent) and cavernous malformation (five percent)], while 75 percent were idiopathic. Case-control analysis revealed no differences between cases and controls in terms of maternal characteristics. Within the investigated intrapartum factors, only foetal distress and emergency caesarean delivery were associated with perinatal haemorrhagic stroke. At the same time, difficult vaginal delivery (forceps or vacuum delivery or shoulder dystocia) did not predict perinatal haemorrhagic stroke (*Armstrong-Wells et al., 2009*). In the

* The first number in parentheses reflects the number of studies with positive results and the second number represents the total number of studies that tested the factor.

same study by Armstrong-Wells et al., gestational age showed a U-shaped relationship: compared with term neonates, premature and postmature infants had an increased risk of perinatal haemorrhagic stroke. Jhawar et al. (2003) studied the risk factors of intracranial haemorrhage in full-term neonates. Their study included all types of intracranial haemorrhage and cases with IPH and SAH (defined as haemorrhagic stroke) constituted 53 percent of the study population. They found that subdural haemorrhage was associated with difficult vaginal delivery (forceps delivery), while IPH was less likely to occur in newborns with difficult vaginal delivery. Lower five-minute Apgar score and resuscitation at birth was associated with an increased risk of intracranial haemorrhage, regardless of haemorrhage type. A platelet count of less than $50x10^9$/L was a strong predictor of IPH compared to other haemorrhage types, with an odds ratio (OR) of 7.0 and a 95 percent confidence interval (CI) that ranged from 1.7 to 29.4, being less than $70x10^9$/L in 31 percent of cases with intraparenchymal haemorrhage (*Jhawar et al., 2003*).

2.1.5. Diagnosis and neuroimaging features of perinatal stroke

There are two reasons why diagnosis of perinatal stroke is based on imaging (*Govaert et al., 2009a*). Firstly, other focal brain lesions must be differentiated from stroke, such as kernicterus, encephalitis, mitochondrial disorders, posterior reversible encephalopathy, tumour, and infarction following hypoglycaemia. Secondly, many stroke cases are detected by routine imaging without acute neurological dysfunction (*Govaert et al., 2009a*). In case of perinatal AIS, the neuroimaging must show a strong suspicion of cerebral artery occlusion. There are two ways of doing this: (1) revealing partial or complete occlusion of the vessel in relation to a focal brain lesion and (2) demonstrating a lesion pattern that can only be explained by occlusion of a specific brain artery (*Govaert et al., 2009a*).

Conventional T2-weighted magnet resonance imaging (MRI), magnetic resonance angiography (MRA) and diffusion-weighted imaging are principal methods for establishing the diagnosis of perinatal AIS (*Raju et al., 2007*). Although a cranial ultrasound is an inexpensive, readily available and portable method that helps detect a stroke in the first few days of life, it is possible to miss more anteriorly or posteriorly located ischemic lesions (*Golomb et al., 2003a; Cowan et al., 2005*). The disadvantages of computed tomography (CT) are radiation and missing early and small lesions.

A focal chronic infarction on neuroimaging (CT or MRI) is obligatory for diagnosing a presumed perinatal stroke (*Kirton et al., 2008*). Such a diagnosis need not be limited to arterial territories, and includes multiple and bilateral infarcts (*Kirton et al. 2008*). Global injuries such as hypoxic-ischemic

encephalopathy, border zone injury (watershed infarction) and bilateral periventricular leukomalacia should be excluded (*Kirton et al., 2008*)

Lesions of neonatal AIS most often occur within middle cerebral artery territories (80 percent), involve cortical structures (92 percent), and are more common in the left hemisphere (57 percent) (*Govaert et al., 2009a; Kirton and deVeber, 2009*). The predilection of the middle cerebral artery involvement may be caused by hemodynamic differences from patent *ductus arteriosus*, transient right to left intracardiac shunt or a more direct route involving the left common carotid (*Benders et al., 2008*). The involvement of multiple arteries in one infant is not uncommon (*Govaert et al., 2009a; Sreenan et al., 2000*).

In preterm infants, AIS is also more common in the left hemisphere (61 percent) and most often within middle cerebral artery territories (81 percent) (*Benders et al., 2008*). Involvement of lenticulostriate branches is characteristic for infants with a gestational age of 28 to 32 weeks, while infants with a gestational age >32 weeks show involvement of the main branch of a cerebral artery (*Benders et al., 2008*).

Focal infarcts are mostly in the territory of the middle cerebral artery (93 percent) also in children with presumed perinatal stroke (*Kirton et al., 2008*). Although subcortical structures are more often affected than in neonatal AIS, the majority of infarcts are still cortical (*Lee et al., 2005; Kirton et al., 2008*). According to recent understanding, most of the subcortical lesions are periventricular venous infarctions (75%) (*Kirton et al., 2008*). Bilateral infarctions are not as common among children suffering from a presumed perinatal stroke as in children with neonatal AIS (*Lee et al., 2005; Golomb et al., 2008*).

Neonatal haemorrhagic stroke includes IPH and SAH (*Govaert et al., 2009a*). It is often difficult to distinguish a primary haemorrhage from an ischemic stroke with secondary haemorrhagic conversion (*Bergman et al., 1985*). Neonatal IPH is typically unilateral and unifocal and lesions are more commonly left-sided, with localisation most often in the parietal or frontal regions (*Armstrong-Wells et al., 2009*). Cerebrovascular imaging is rarely performed in the acute phase of haemorrhage and, when it is performed, vascular pathologies have been seldom detected in case series or population-based studies (*Bergman et al., 1985; Sandberg et al., 2001; Armstrong-Wells et al., 2009*). Congenital arterial aneurysm have been reported as the cause of neonatal IPH or SAH in case reports since 1949 (*Vizcaíno-Díaz et al., 2009; Van Raay et al., 2009, González-Bonet et al., 2010*). Arteriovenous malformation is a rare cause of neonatal intracranial haemorrhage, among which those involving the vein of Galen are the most common (*Volpe, 2008*).

2.1.6. Outcome of perinatal stroke

Children with perinatal stroke develop long-term disabilities including motor deficits, epilepsy and cognitive disorders. Perinatal stroke is a common cause of congenital hemiparesis, the severity of which ranges from mild hand weakness to hemiplegia or even quadriplegia in children with bilateral stroke (*Lynch et al., 2009*). Motor deficit occurs in 37–60 percent of children who suffer from neonatal AIS (*Trauner et al., 1993; Sreenan et al., 2000; Lee et al., 2005; Golomb et al., 2008b*). In children with presumed perinatal stroke, the prevalence of motor deficit is even higher, ranging from 82 to 91 percent of children (*Lee et al., 2005; Golomb et al., 2008b*); this is partly because retrospective diagnosis confers a selection bias (*Kirton et al., 2008*). In outcome studies, most children (95 percent) with neonatal unilateral ischemic stroke and 67 percent of children with bilateral infarction will walk (*Golomb et al., 2003b*).

Several motor outcome predictors following perinatal AIS have been discovered. Poor motor outcome of neonatal AIS has been associated with the presence of prothrombotic risk factor (*Mercury et al., 2001*), an abnormal electroencephalogram (EEG) background pattern (*Mercury et al., 1999*) and male gender (*Golomb et al., 2008b*). Lesions that concomitantly involve the motor cortex, the internal capsule and the basal ganglia are associated with later hemiparesis (*Mercury et al., 1999; Mercury et al., 2004*). A study of neonatal stroke and presumed perinatal stroke found that several radiological characteristics were associated with an increased risk for motor and language disability such as the size of stroke area, injury to Broca's or Wernicke's area, and injury to the internal capsule or basal ganglia (*Lee et al., 2005*).

Rates of epilepsy after perinatal stroke depend partly on when it is evaluated. Epilepsy develops in 37–67 percent (*Lee et al., 2005; Golomb et al., 2007*) of children with neonatal AIS and in 23–42 percent of patients with presumed perinatal stroke (*Golomb et al., 2001; Lee et al., 2005; Kirton et al., 2008; Fitzgerald et al., 2007*). Complex partial seizures are most common type of epilepsy developed after perinatal ischemic stroke (*Golomb et al., 2001*). The median age of epilepsy onset is 16 months (range: seven months to 10.3 years). According to the study by Golomb et al. (2007), neonatal seizures do not predict later epilepsy.

The outcome of neonatal haemorrhagic stroke is not thoroughly studied. Neurological outcome is normal in 36 percent of children with neonatal IPH, while other children have either delayed speech, hemiparesis or cognitive delay at outcome (*Sandberg et al., 2001*).

Recurrent symptomatic thromboembolism occurs in 3.3 percent of children who suffer from neonatal AIS being lower beyond infancy (*Kurnik et al., 2003*).

2.2. Childhood arterial stroke

2.2.1. Definition and classification

Childhood stroke is a cerebrovascular event that occurs between one month and 18 years of age (*Lynch et al., 2002; Amlie-Lefond et al., 2008*). Childhood arterial stroke is further subdivided into ischemic and haemorrhagic stroke, whereas haemorrhagic stroke includes IPH and SAH (*Amlie-Lefond et al., 2008*). Ischemic stroke constitutes around 60 percent of all childhood strokes (*Kleindorfer et al., 2006*). Most cases of haemorrhagic stroke (82 percent) are IPH, with SAH only constituting a minority (18 percent) (*Jordan et al., 2009*).

Like perinatal stroke, the diagnosis of childhood stroke is based on neuroradiological confirmation, which is opposite to adult stroke. The main reasons for this are often the unspecific presentation of childhood stroke and high frequency of mimics of stroke in childhood. Although a stroke is defined as a neurological deficit lasting at least 24 hours, many children with a transient ischemic attack (TIA) with symptoms that last ≤ 24 hours have a recent cerebral infarction/haemorrhage on imaging; these have sometimes been included in childhood stroke studies (*Pappachan and Kirkham, 2008*).

2.2.2. Epidemiology of childhood stroke

Although the number of studies on childhood stroke has increased markedly in recent decades, there are still not many studies on its incidence rate. The rate may have increased over the past decades due to increased recognition, the possibilities of modern neuroradiology and therapeutic advances that allow children with predisposing conditions to survive (*Pappachan and Kirkham, 2008; Mallick and O'Callaghan, 2010*). Previous studies with variable inclusion criteria have found annual incidence rates of childhood stroke that range from 1.3 to 2.7 per 100,000 children. The exceptions are studies from France (*Giroud et al., 1995*) and the USA (*Zahuranec et al., 2005*), which reported significantly higher incidence rates of 4.3–13.0/100,000 (see Table 2). The reported annual incidence rate of childhood AIS has varied from 0.6 to 7.9 and haemorrhagic stroke ranges from 0.7 to 5.1 per 100,000 children (see Table 2). The background prevalence of sickle cell disease, moyamoya disease or infections (meningitis) may be important factors that influence the epidemiology in different countries (*Mallick and O'Callaghan, 2010*). There are few studies of the epidemiology outside North America and Western Europe (Table 2).

TIA is seldom included in epidemiological studies (*Zahuraenc et al., 2005*) and data on the incidence of TIA in children is still missing.

Table 2. Incidence rates of childhood stroke in previous studies

Source	Country	Age Range	No of Patients	Incidence rate		
				Total	AIS	HS
Schoenberg, 1978	USA	0–15yr	69	2.5	0.6	1.9
Eeg-Olofsson, 1983	Sweden	6mo–16yr	5	2.1	+	+
Broderick, 1993	USA	3wk–15yr	16	2.7	1.2	1.5
Giroud, 1995	France	0–16yr	28	13.0	7.9	5.1
Earley, 1998	USA	1–15yr	35	1.3	0.6	0.7[a]
Fullerton, 2003	USA	1mo–20yr	2278	2.3	1.2	1.1
Chung, 2004	China	1mo–16yr	50	2.1	+	+
Barnes, 2004	Australia	0–20yr	95	–	1.8	–
Steinlin, 2005	Switzerland	0–16yr	80	2.1[b]	–	–
Zahuranec, 2005	USA	1mo–20yr	8[c]	4.3	1.1	3.2
Fullerton, 2007	USA	0–20yr	181	–	2.0[d]	–
Fullerton, 2007	USA	0–20yr	153	–	–	1.7
Simma, 2007	Austria	1mo–19yr	22	2.7	1.96	0.75
Jordan, 2009	USA	0–20yr	116	–	–	1.4

HS, haemorrhagic stroke; [a]SAH was excluded; [b]29 percent were neonatal stroke patients; [c]one patient with TIA, [d]46 percent were neonatal stroke patients; + means included; – means excluded

Ten of the 14 published studies (71 percent) have been retrospective and most of the rest have been based on stroke registry (*Giroud et al., 1995; Chung et al., 2004; Steinlin et al., 2005*). Multitiered case ascertainment suggested by Sudlow and Wardlow (1996) (for example, research of hospital discharge diagnoses, outpatient diagnoses, electronic neuroimaging reports, autopsy reports or cross-reference with previous studies) was used in seven studies (*Schoenberg et al., 1978; Broderick et al., 1993; Giroud et al., 1995; Fullerton et al., 2003; Fullerton et al., 2007a; Fullerton et al., 2007b; Jordan et al., 2009*).

Childhood AIS and IPH occur most frequently (29 percent) among infants (children >1 month and <1 year old), whereas children aged 15 through 19 have the highest rates of SAH (*Fullerton et al., 2003; Kleindorfer et al., 2006*). This could explain the lowest reported incidence rate for childhood stroke in the study by Earley et al. (1998), because they excluded children under one year of age.

Most of the population-based studies of childhood stroke have reported male predominance. The International Paediatric Stroke Study of 1187 children, indicating a male to female ratio of 1.5:1 (*Golomb et al., 2009*), supported this finding. In addition, male predominance (56–63 percent) is often recorded among patients with haemorrhagic childhood stroke (*Meyer-Heim et al., 2003; Jordan et al., 2009; Lo et al., 2008*). The reason for the increased risk of stroke among male children is unknown. Possible reasons for this include the protective role of oestrogen in females, behavioural differences and the more resistant response to hypoxia/ischemia by female cells of the central nervous system (*Mallick and O'Callaghan, 2010*). There is evidence that gender

differences of paediatric ischemic stroke are associated with elevated endogenous testosterone concentrations. A recent study by Normann et al. (2009) showed that testosterone levels above the 90th percentile for age and gender were detected in 14 percent and 19 percent of children with AIS and SVT, respectively, compared to 1.8 percent of control children (P=0.002). Testosterone supports the intristic hypercoagulable system, in contrast to the supportive role of oestrogen for the intristic anticoagulation system (*Normann et al., 2009*).

Unlike paediatric AIS, TIA occurs more frequently in girls and in older children (*Herak et al., 2009*). According to a study by Fullerton et al. (2003), black children have twice the increased risk for childhood stroke than white children. The same study revieled that black children were at increased risk even when cases of sickle cell disease were excluded.

According to the National Center for Health Statistics in the United States, mortality rates for paediatric stroke are 0.09/100,000 person-years for ischemic stroke, 0.14 for IPH and 0.11 for SAH (*Mallic and O'Callaghan, 2009*). Haemorrhagic stroke account for 74 percent of deaths from paediatric stroke (*Fullerton et al., 2002*). Children under one year old have the highest mortality rate and the male mortality rate is higher than the female rate (*Fullerton et al., 2002; Mallick et al., 2010*). Previous studies have found 30-day case-fatality among patients of childhood AIS of 4–16 percent (*Lanthier et al., 2000; Broderick et al., 1993; Fullerton et al., 2003; Fullerton et al., 2007a*). Case-fatality among childhood haemorrhagic stroke patients varied between 20 percent and 34 percent in previous studies (*Broderick et al., 1993; Lanthier et al., 2000; Fullerton et al., 2007a; Lo et al., 2008*).

2.2.3. Clinical presentation of childhood stroke

Childhood AIS most commonly presents with acute hemiparesis. Other common presenting features are aphasia, visual disturbance, seizures or altered level of consciousness (*Giroud et al., 1997*). Seizures are much more commonly associated with childhood stroke than in adults (*Chadehumbe et al., 2009*). Stroke in the posterior circulation can present as ataxia, vertigo or vomiting (*Kirkham et al., 2004*). Seizures and altered mental status are more common and focal weakness is less common in children aged less than one year than in older children (*Zimmer et al., 2007*). The mode of symptom onset in approximately half of the children with AIS may be non-abrupt (*Braun et al., 2007*). In these cases, neurological symptoms progress smoothly and reach maximum severity in more than 30 minutes, or the course of symptoms fluctuates. The non-abrupt onset of symptoms is strongly associated with arteriopathic AIS (OR=5.6; 95% CI: 1.6–19.4) (*Braun et al., 2007*).

Headache (46–77 percent), vomiting (21–59 percent), impaired consciousness (16–50 percent), seizures (26–41 percent) and focal neurological deficits

(13–50 percent) are characteristic of IPH (*Al-Jarallah et al., 2000; Mayer-Heim et al., 2003; Beslow et al., 2010*). Acute life-threaten intracranial hypertension occurs in 45 percent of children with IPH (*Beslow et al., 2010*), while focal neurological deficits are most common among children of at least six years of age (*Lo et al., 2008*).

2.2.4. Risk factors of childhood stroke

Many disorders are associated with childhood stroke in case series but the pathogenesis of childhood stroke is often unclear. Unlike adult brain infarct, childhood AIS has a wide range of risk factors. An underlying predisposing medical condition is found in approximately half of the children (*Ganesan et al., 2003; Pappachan and Kirkham, 2008*). After examinations, a possible etiological factor (i.e., cardiac pathology, arteriopathy and/or prothrombotic disorder) can be found in most children who were previously considered healthy (*Ganesan et al., 2003; Pappachan and Kirkham, 2008*) and most patients with childhood stroke have more than one risk factor (*Simma et al., 2007*). However, in spite of thorough investigations, the reason for childhood stroke remains unknown in 10–15 percent of children (*Barreirinho et al., 2003; del Balzo et al., 2009*).

Vasculopathy is the most common primary or secondary final pathway for childhood AIS. Abnormalities of the cerebral arteries are found in up to 80 percent of children whom vascular investigations are performed (*Ganesan et al., 2003; Simma et al., 2007*). Arteriopathy is defined as "any abnormality on cerebrovascular imaging except isolated vessel occlusion which may represent an embolus rather than a primary disorder of the blood vessel" (*Amlie-Lefond et al., 2009*). The most common form of arteriopathies in childhood AIS is recently defined focal cerebral arteriopathy (*Amlie-Lefond et al., 2009*). Focal cerebral arteriopathy is a "stenosis on vascular imaging not otherwise classified as dissection, moyamoya, sickle cell arteriopathy, post-varicella arteriopathy, vasculitis, or other specific diagnoses" (*Amlie-Lefond et al., 2009*). A stenosis of a cerebral artery can be found in 53–64 percent of children with AIS on cerebral vascular imaging (*Ganesan et al., 2003, Amlie-Lefond et al., 2009*). Focal cerebral arteriopathy includes the term "transient cerebral arteriopathy" (*Sebire et al., 2004*) but excludes post-varicella arteriopathy (*Lanthier et al., 2005*). Other common forms of arteriopathies predisposing childhood AIS are moyamoya, sickle cell arteriopathy, systemic vasculitis and dissection of the cervical arteries (*Amlie-Lefond et al., 2009*). Arteriopathy is a major risk factor for recurrent stroke (*Sträter et al., 2002, Fullerton at al., 2007a*).

Pre-existing congenital or acquired cardiac diseases are present in up to 30 percent of childhood AIS cases (*Steinlin et al., 2005; Barnes et al., 2004a*). Cardiac surgery, cardiac catheterisation and immobilisation are additional factors associated with cardiac disease and increased risk for AIS. The risk of

ischemic stroke is 5.4 per 1000 children undergoing a cardiac operation (*Domi et al., 2008*). The role of paradoxical embolism through a patent foramen ovale as a risk factor for childhood AIS remains arguable. Moyamoya is associated with congenital heart disease and should be considered both in non-syndromic and syndromic (Down's syndrome, Williams syndrome, or neurofibromatosis type 1) patients with congenital heart disease (*Lutterman et al., 1998*).

Infection and inflammation are triggers for childhood AIS (*Dlamini and Kirkham, 2009*). The final causal role of infection and inflammation is difficult to prove because of the rarity of strokes and the high prevalence of infectious diseases in children (*Kirkham et al., 2000; Lindsberg and Grau, 2003*). Various mechanisms have been suggested for the role of infections and inflammation in the pathogenesis of stroke (*Lindsberg and Grau, 2003*). Childhood AIS have been associated with upper respiratory tract infections, ear-nose-throat infections, meningitis, encephalitis, *Varicella zoster* virus, *Mycoplasma pneumoniae*, Ebstein-Barr virus infection and tuberculosis (*Barnes et al., 2004; Steinlin et al., 2005; Shi et al., 2008; Wang et al., 2009; Rasul et al., 2009*). Infections precede a significant proportion of AIS in childhood with reported prevalence varying from 11 to 48 percent (*Barnes et al., 2004; Steinlin et al., 2005; Fullerton et al., 2007a; Shi et al., 2008; Wang et al., 2009; Rasul et al., 2009*). Arteriopathy appears to be associated with fever and infection in children with AIS (*Amlie-Lefond et al., 2009; Braun et al., 2009*). When *Varicella* infection precedes the onset of AIS by less than 12 months, the term 'post-varicella arteriopathy' is used (*Lanthier et al., 2005*). Varicella may proceed within 12 months up to 31 percent of childhood strokes and in most of the cases (83–91 percent). Brain vascular investigations performed after a stroke reveal areas of stenosis in the proximal portion of the major cerebral arteries (*Lanthier et al., 2005; Askalan et al., 2001*). The most plausible mechanism for *Varicella* causing AIS involves intraneuronal migration of the *Varicella zoster* virus from the trigeminal ganglion and nerve to the cerebral arteries (*Askalan et al., 2001*).

Sickle cell disease has a high risk for childhood AIS, given that stroke is 250 times more common among children with sickle cell disease than in the general paediatric population (*Seidman et al., 2007*). Eleven percent of patients with sickle cell disease have had stroke by the age of 20 (*Ohene-Frempong et al., 1998*). The sickled cells have increased adhesion to endothelium, which results in the formation of a thrombus. Sickle cell disease is the only condition that has proven method for primary stroke prevention. Chronic blood transfusion therapy results in an over 80 percent reduction in the risk of stroke (*Fullerton et al., 2004*). Sickle cell disease compromises up to 36 percent of the risk factors of childhood stroke in cohort studies from Equatorial countries (*Emam et al., 2009*) and up to 20 percent in multiracial population-based studies from the United States (*Earley et al., 1998*). However, it is low in studies from Europe.

Trauma is a known risk factor for childhood ischemic stroke, and blunt trauma to the posterior pharynx, cervical spine rotation/dislocation and dissection has been suggested as a possible underlying mechanism (*Kirkham et*

al., 2004). Both cervical and intracerebral dissections of arteries are associated with trauma and have been diagnosed in 9–20 percent of cases in hospital series (*Chabrier et al., 2000; Lee et al., 2010*). The preceding trauma can be mild or significant or there could be no trauma in anamnesis in children with dissection (*Fullerton et al., 2001; Lee et al., 2010*). Intracranial dissections are more common among children with stroke than they are in adults and children complain of pain more rarely than adults (*Fullerton et al., 2001*). The mechanism is typically an embolism that results from the migration of a thrombus that is originally located at the site of subintimal dissection (*Amlie-Lefond et al., 2008*). There are descriptions of a series of young children (one to seven years) with acute hemiparesis caused by striatocapsular infarction following mild head trauma (*Kieslich et al., 2002; Shaffer et al., 2003*). The proposed mechanism is the disruption of the lenticulostriate branches of the cerebral arteries between the mobile extracerebral portion and the fixed intracerebral portion (*Shaffer et al., 2003*).

Although thrombophilias have received considerable attention in childhood stroke studies, the importance of thrombophilia in childhood stroke pathogenesis is still unknown. Thrombophilias remain mild risk factors with ORs between four and 10, and results vary from study to study (*Amalie-Lefond et al., 2008*). The reported prevalence of thrombophilia varies in population-based studies from 7–50 percent of the childhood AIS cases *(Fullerton et al., 2007a; Simma et al., 2007*). The two most comprehensively studied inherited prothrombotic disorders are FVL and the PT 20210G>A mutation. A single point mutation (G to A transition in position 1691) in the factor V gene leads to Arg506Gln amino acid exchange in protein. This change determines a resistance to a physiological anticoagulant, activated protein C (*Kalafatis et al., 1994*). Prothrombin is the precursor of the serine protease thrombin that converts fibrinogen into fibrin. A single-nucleotide G to A transition in 3'-untranslated region of prothrombin gene at position 20210 is associated with increased prothrombin levels (*Poort et al., 1996*). Many studies have tested the association between FVL and PT 20210G>A mutation and childhood AIS. The study groups are usually small and the results have been contradictory. Eight out of 18 studies have found a significant association between FVL and childhood AIS (*Zenz et al., 1998; Akar et al., 1999; Nowak-Göttl et al., 1999; Kenet et al., 2000; Akar et al., 2001; Duran et al., 2005; Komitopoulou et al., 2006; Herak et al., 2009*). Only two of 11 studies have found a significant association between PT 20210G>A mutation and childhood AIS (*Akar et al., 1999; Nowak-Göttl et al., 1999*).

Other prothrombotic factors that have been associated with childhood AIS in case-control studies are hyperhomocysteinemia (3/4)[*] and the TT677 genotype of methylenetetrahydrofolate reductase (MTHFR) gene (1/13), deficiencies of

[*] The first number in parentheses reflects the number of studies with positive results and the second number reflects the total number of studies that tested the factor.

protein C (2/4), increased levels of anticardiolipin antibodies (3/5), lipoprotein (a) >30 mg/dL (2/2), and thrombomodulin (1/1), increased plasminogen activator inhibitor-1 activity (1/1), H2 haplotype of plasma glutathione peroxidase gene (1/1) and tissue factor pathway inhibitor concentrations below the 10th age-dependent percentiles (1/1) (*van Beynum et al., 1999; Nowak-Göttl et al., 1999; Sträter et al., 1999; Kenet et al., 2000; Nowak-Göttl et al., 2001; Duering et al., 2004; Pilarska et al., 2006; Sirachainan et al., 2006; Voetsch et al., 2007*). Deficiencies of protein S and antithrombin III (*Nowak-Göttl et al., 1999; Duran et al., 2005*), the CC1298 genotype of MTHFR gene (*Komitopoulou et al., 2006; Akar et al., 2001; Morita et al., 2009*), and the 4G/5G genotype in the promoter of the plasminogen activator inhibitor-1 gene (*Nowak-Göttl et al., 2001; Komitopoulou et al., 2006; Ozyrek et al., 2007*) have not been found to have a significant association with childhood AIS in case-control-studies.

It is increasingly accepted that the coexistence of several prothrombotic factors or trombophilia with other risk factors is needed, rather than a single prothrombotic factor, to develop strokes in children (*Amlie-Lefond et al., 2008*). Multiple risk factors are found in 31 percent of the childhood AIS cases and the recurrence risk of childhood AIS is increased in children with multiple risk factors (*Lanthier et al., 2000*).

The research devoted to childhood haemorrhagic strokes has been scant. According to the pooled data by Jordan and Hillis (2007), which included 500 children with haemorrhagic stroke diagnosed between 1965 and 2004, the main aetiologies of childhood IPH were arteriovenous malformation (39 percent), cavernoma (11 percent), aneurysm (nine percent), haematological abnormality (21 percent), and brain tumour (six percent). More recent studies have analysed the aetiology of childhood IPH with contradictory results. Beslow et al. (2010) and Ribaupierre et al. (2008) both found that vascular malformations are detected even more frequently (87–91 percent of children with IPH) than in previous studies. Their results found arteriovenous malformation in 55 percent, cavernoma in 14–32 percent and aneurysm in 4.5–18 percent of cases. In the cohort of Lo et al. (2008), vascular lesions accounted for only 34 percent of cases, while the proportion of brain tumours (18 percent) and congenital heart disease with or without surgery (10 percent) were larger than previously reported. According to Lo et al. (2008), the proportion of brain tumours and congenital heart disease should be markedly increased among children with intracranial haemorrhage due to the widespread use of contemporary imaging techniques and improved care of chronic illnesses.

Aneurysm is the most common vascular malformation (71 percent) causing SAH (*Jordan et al., 2009*).

Haematological abnormalities are more commonly detected in Eastern countries and in rural areas, where not all newborns receive vitamin K at birth. In China, for example, haematological abnormalities are a major etiologic factor for childhood haemorrhagic stroke (64–77 percent) (*Chung et al., 2004; Wang*

et al., 2009). In contrast, haematological abnormalities account for only 15 percent of cases in Western countries (*Al-Jarallah et al., 2000*). The aetiology of IPH remains unclear in 9–17 percent of cases (*Jordan and Hillis, 2007; Lo et al., 2008; Beslow et al., 2010*).

2.2.5. Diagnosis of childhood stroke

In children, clinical presentation consistent with acute stroke may have a non-vascular aetiology in a 21–29 percent of cases (*Ganesan et al., 2003; Shellhaas et al., 2006*). Brain imaging is therefore important for distinguishing between vascular and non-vascular aetiologies for acute focal deficits (*Mallick and Ganesan, 2008*). The main differential diagnosis of childhood AIS includes hemiplegic migraine, postictal Todd's paresis, acute disseminated encephalomyelitis, reversible posterior leukoencephalopathy syndrome, focal encephalitis and metabolic stroke (*Pappachan and Kirkham, 2008*). Because the initial imaging is often negative for true stroke, head MRI with diffusion-weighted imaging is frequently required to differentiate a childhood stroke from stroke mimic (*Amlie-Lefond et al., 2008*).

MRI is more sensitive than CT for detecting AIS, particularly in the case of acute ischemia and posterior fossa lesions (*Amlie-Lefond et al., 2008*). Diffusion-weighted imaging can detect cerebral ischemia within minutes of onset, as cytotoxic oedema results in decreased apparent diffusion coefficient, which will be hyperintense on a diffusion-weighted image. Perfusion-weighted imaging shows areas of decreased cerebral perfusion. Areas with normal diffusion but reduced perfusion (perfusion/diffusion mismatch) might be at-risk tissue that has not yet infarcted. Patients with mismatch benefit mostly from early reperfusion strategies according to adult stroke studies (*Albers et al., 2006*). One main disadvantage of CT and especially MRI studies is that they require sedation of infants and young children or uncooperative patients.

MRA adds information about the cervical and intracerebral arteries. Vascular imaging is important because of the high prevalence of underlying cerebral arteriopathy in patients with AIS. MRA is as good as conventional angiography at detecting pathologies in the internal carotid artery and the middle cerebral artery but is less sensitive to detect small vessel abnormalities (*Husson et al., 2002; Benseler et al., 2006*). Although the importance of small vessel disease in childhood stroke is unknown, it has been claimed that isolated involvement of small arteries is a rare cause of arteriopathy in childhood stroke (*Husson et al., 2002*). The main advantages of MRA main are that there is no exposure of radiation and no requirement for intravenous access. However, MRA may overestimate the stenosis (*Husson et al., 2002*). Conventional catheter angiography is still the gold standard for diagnosing cerebral arteriopathy. Conventional catheter angiography is an invasive procedure with potential risk of iatrogenic vessel injury or thromembolic event. However, this

method has had a low (0–0.4 percent) complication rate (local complications only) in children (*Burger et al., 2006; Fung et al., 2005*).

Childhood AIS usually involves the middle cerebral artery territory and subcortical infarction is common. Strokes in the anterior circulation are more common than in the posterior circulation (*Amlie-Lefond et al., 2009*). Focal stenosing arteriopathy and post-*varicella* arteriopathy locate infarct in the basal ganglia and infarcts are limited to anterior circulation (*Askalan et al., 2001; Braun et al., 2009*).

CT is the initial imaging study of choice for haemorrhagic stroke (*Broderick et al., 2007*) because it is rapid, widely available and clearly distinguishes between haemorrhagic and ischemic stroke (*Broderick et al., 2007*). Susceptibility-weighted MRI sequences can clearly identify haemorrhage but this method is not universally available (*Jordan and Hillis, 2007*). Vascular imaging is important for identifying any structural lesion that may predispose to recurrence and may require additional treatment. CT angiography can be combined with the initial CT examination but this combination is limited by CT's poor delineation of parenchymal pathology (*Liu et al., 2006*). Liu et al. (2006) found that the combination of MRI/MRA identified the cause of IPH in 66 percent of children, while conventional catheter angiography alone identified it in 61 percent of children. The limitations of MRI/MRA are the inability to detect subtle pathology or pathology affecting small vessels or to provide adequate hemodynamic information (*Liu et al., 2006*). On the other hand, conventional catheter angiography is not able to detect cavernous angiomas and tumours, which are visible on MRI images (*Liu et al., 2006*). CT angiography is the first line of investigation for children (and adults) presenting with SAH (*Liu et al., 2006*).

IPH is supratentorial in 82–91 percent of patients (*Lo et al., 2008; Ribaupierre et al., 2008; Beslow et al., 2010*). Intraventricular extension is common, occurring in up to half of cases (*Mayer-Heim et al., 2003; Beslow et al., 2010*).

2.2.6. Delay in diagnosis of childhood arterial ischemic stroke

Early diagnosis of AIS is a challenge for physicians but is a prerequisite for "hyperacute" treatment options like thrombolysis and neuroprotective strategies. Studies from the USA, the United Kingdom, Canada and Australia have shown that childhood stroke is rarely diagnosed within three (7–10 percent) or six (20–33 percent) hours from symptom onset (*Gabis et al., 2002; McGlennan and Ganesan, 2008; Rafay et al., 2009a; Srinivasan et al., 2009*).

The median interval from symptom onset to diagnosis of AIS ranges from 20 to 22.7 hours (*Gabis et al., 2002; Rafay et al., 2009a; Srinivasan et al., 2009*).

Prehospital delay (from first symptom to first assessment) varies from one to nine hours, while in-hospital delay (from arrival at a hospital until the diagnosis of AIS) is estimated at 12.7 hours (*Gabis et al., 2002; Rafay et al., 2009a; Srinivasan et al., 2009*). Only 58 percent of children reach the hospital within three hours and 69 percent reach it within six hours (*Rafay et al., 2008*). In only 26–38 percent of children is stroke considered by an emergency doctor (*Rafay et al., 2009a)* and in 58 percent of cases AIS is diagnosed by a child neurologist at the first assessment (*Braun et al., 2006*). The other problem is that the initial neuroimaging (most commonly CT) diagnose AIS in only 53 percent of cases (*Rafay et al., 2009a*).

Probable reasons for this delay include the lack of clinical suspicion of stroke in children and the frequency of stroke mimics in children (*Amlie-Lefond et al., 2008*). Another reason may be the subacute onset of symptoms. According to Rafay et al. (2009), predictors of longer total delay are young age, parent's help-seeking action, non-ambulance transport, lower stroke severity, non-abrupt onset and the absence of altered consciousness.

2.3. Paediatric sinovenous thrombosis

2.3.1. Definition of paediatric sinovenous thrombosis

SVT is rare in children but increasingly recognised because of the increased awareness, improved neuroradiological techniques, and increased survival of children and neonates with diseases predisposing to SVT. SVT occurs in foetuses (*Laurichesse et al., 2008*), in preterm (*Bassan et al., 2006*) and term neonates, as well as in older neonates and children (*deVeber et al., 2001; Heller et al., 2003*). Modern understanding suggests that periventricular haemorrhagic infarction in preterm newborns, which was earlier termed grade IV germinal matrix intraventricular haemorrhage, is a venous haemorrhagic infarction in the drainage area of the periventricular terminal vein (*Volpe, 2001*).

2.3.2. Epidemiology of paediatric sinovenous thrombosis

There are few epidemiological studies on paediatric SVT. Heller et al. (2003) estimated the annual incidence rate of SVT to be 2.6 per 100,000 in neonates and 0.35 per 100,000 in older children. DeVeber et al. (2001) reported the annual incidence rate of paediatric SVT to be 0.67 per 100,000 children. Forty-three percent of the subjects in their study were neonates. If incidence rates were calculated separately for childhood stroke, the incidence rate for childhood SVT would be 0.38/100,000, which is similar to the result achieved by Heller et al. (2003).

Population-based studies report that newborns account for 27–43 percent of the total paediatric SVT (*deVeber et al., 2001; Heller et al., 2003*) and 54 percent of the patients are aged less than one year (*deVeber et al., 2001*). Several studies report male predominance (male: female ratio 1.2:1 to 2.5:1) among paediatric SVT patients (*deVeber et al., 2001; Carvalho et al., 2001; Bonduel et al., 2006*). Unilateral periventricular venous infarction occurs in one percent of preterm infants with birth weight less than 2500 grams and the incidence of periventricular venous infarction increases as the birth weight decreases (*Bassan et al., 2006*).

Mortality rate of 10–27 percent among paediatric SVT patients have been reported (*Gentilomo et al., 2006*).

2.3.3. Clinical presentation of paediatric sinovenous thrombosis

Clinical features of SVT vary and are often non-specific. The main symptoms and signs of childhood SVT are headache, vomiting, seizures, hemiparesis, decreased level of consciousness, papilloedema and cranial nerve palsy (*deVeber et al., 2001; Wasay et al., 2008; Mallick et al., 2009*). Fever may be present in 29–56 percent of children with SVT (*Carvalho et al., 2001; Barnes et al., 2004b; Wasay et al., 2008; Sébire et al., 2005; Mallick et al., 2009*). In 14 percent of the cases, the clinical presentation may be subacute with chronic headache, vomiting, lethargy, anorexia and drowsiness for three weeks or more (*Sébire et al., 2005*).

Newborns present more frequently with seizures and less frequently with neurological signs than older infants and children (*deVeber et al., 2001*). Two-thirds of neonates with SVT present early (within 48 hours) and one-third present late, between 48 hours and 28 days (*Nwosu et al., 2008*). Early presentation may be confounded by comorbidities and acute illness and symptoms are non-specific (*Ramenghi et al., 2009*). Respiratory distress, hypoxia and poor tone are characteristic of early presentation in neonates (*Nwosu et al., 2008*). Late presentation is more often associated with neurological symptoms such as seizures, lethargy, apnoea and poor feeding (*Ramenghi et al., 2009*). Seizures occur in 55–79 percent of neonates with SVT and are not associated with the time of presentation (*Fitzgerald et al., 2006; Nwosu et al., 2008; Jordan et al., 2010*). In the case of extensive SVT, a tense anterior fontanel together with dilated scalp veins may be present.

Some children and newborns with SVT have no neurological disturbances and are diagnosed accidentally by screening neuroimaging (*Heller et al., 2003; Fitzgerald et al., 2006; Nwosu et al., 2008*).

2.3.4. Risk factors of paediatric sinovenous thrombosis

The main predisposing factors for paediatric SVT are (1) head and neck disorders (infection and others), (2) acute systemic illness (sepsis and dehydration), (3) chronic disease predisposing to SVT (cardiac disease, surgical procedures, nephrotic syndrome, systemic lupus erythematosus, brain tumour) and (4) haematological disorders (congenital and acquired prothrombotic disorders and anaemia) (*deVeber et al., 2001*). Among local head and neck infections, otitis, mastoiditis, and meningitis precede SVT in 38–62 percent of cases (*deVeber et al., 2001; Mallick et al., 2009*). Anaemia may be present in up to 52 percent of paediatric SVT patients (*Sébire et al., 2005*). Increased blood viscosity due to poorly deformable erythrocytes, reactive thrombocytosis and elevated erythropoietin levels associated with iron deficiency anaemia may predispose to SVT (*Benedict et al., 2004*).

A previously known underlying co-morbid condition (head and neck disorders, acute systemic illness, chronic systemic illness) is present in most (82–100 percent) children with SVT (*deVeber et al., 2001; Barnes et al., 2004b; Kenet et al., 2004; Mallick et al., 2009*). After laboratory investigations, prothrombotic factors are found among the remaining cases (*Kenet et al., 2004; Mallick et al., 2009*). The main risk factors in the neonatal period are peri-partum complications, dehydration and comorbid medical condition (*deVeber et al., 2001; Wu et al., 2002; Fitzgerald et al., 2006*). Risk factors are found in most (96 percent) neonates with SVT (*Jordan et al., 2010*).

Prothrombotic disorders are found in 32–62 percent of children with paediatric SVT (*deVeber et al., 2001; Heller et al., 2003; Sébire et al., 2005; Kenet et al., 2004; Bonduel et al., 2006*) and occur more often among patients without co-morbid conditions (*Kenet et al., 2004*). At least six case-control studies have studied the association between prothrombotic factors and pae-diatric SVT (*Hagstrom et al., 1998; Bonduel et al., 2003; Heller et al., 2003; Kenet et al., 2004; Bonduel et al., 2006; Morita et al., 2009*). Heller et al.'s (2003) study showed that deficiencies of protein C and protein S, FVL, and increased lipoprotein (a) level >30mg/dl have a significant association with paediatric SVT on univariate analysis. In a multivariate analysis, only elevated lipoprotein (a), and protein C deficiency retained their statistically significant association with SVT (*Heller et al., 2003*). Multivariate analysis also revealed that the coexistence of an underlying predisposing condition and prothrombotic factor predicted SVT in children (OR=3.9; 95% CI: 1.8–8.6). Patients who are heterozygous for the PT 20210G>A mutation have four times greater risk of recurrent venous thrombosis (OR=4.3; 95% CI: 1.1–16.2) (*Kenet et al., 2007*).

2.3.5. Neuroimaging of paediatric sinovenous thrombosis

The two major goals of radiological diagnosis of SVT are imaging of the thrombus and the associated cerebral lesions such as haemorrhage due to venous infarction (*Ramenghi et al., 2009*). In neonates, colour flow Doppler ultrasonography is useful for identifying thrombosis in the saggital sinus and left transverse sinus (*Ramenghi et al., 2009*). A non-contrast CT may show hyperdensity of the sinuses (the cord sign = hyperdense thrombosed veins) (*Renowden, 2004*). CT with contrast highlights filling defects in the sinuses (the delta sign = a filling defect in the superior saggital sinus) (*Renowden, 2004*). CT venography provides more accurate evaluation of the venous sinuses and may improve diagnostics when MRI is not available (*Ramenghi et al., 2009*). Although MRI is excellent for providing detailed information of parenchymal lesions, it is difficult to interpret signals in sinuses. Caution is needed as the slow-flow signal can be misdiagnosed as no-flow signal (*Ramenghi et al., 2009*). CT may miss 16–31 percent of paediatric SVT cases confirmed by MRI (*deVeber et al., 2001; Mallick et al., 2009*).

In most cases (76–86 percent), the thrombosis is located superficially (*deVeber et al., 2001; Heller et al., 2003; Sébire et al., 2005*). The transverse sinus, the superior saggital sinus and the straight sinus are most common locations of paediatric SVT (*Teksam et al., 2008*). Multiple sinuses are involved in 49–70 percent of children (*deVeber et al., 2001; Fitzgerald et al., 2006; Wasay et al., 2008*).

Cerebral parenchymal infarcts are present in 41–60 percent of children (*deVeber et al., 2001; Sébire et al., 2005; Teksam et al., 2008*). Infarction is the result of persistent regional increase of venous pressure exceeding regional arterial perfusion pressure (*Ramenghi et al., 2009*). Most of the infarcts are haemorrhagic (*deVeber et al., 2001; Wasay et al., 2008; Ramenghi et al., 2009*). Purely ischemic infarcts may be caused by local reduction of arterial inflow due to arteriolar constriction in response to downstream venous flow impairment (*Ramenghi et al., 2009*). Neonates tend to have haemorrhagic infarcts more frequently than older children (*deVeber et al., 2001; Carvalho et al., 2001; Fitzgerald et al., 2006; Teksam et al., 2008*). This may be caused by a deficiency of the protective mechanisms (e.g., opening of reserve capillaries) due to immaturity (*Teksam et al., 2008*). Extraparenchymal haemorrhage is present in nine percent of children (*deVeber et al., 2001*). Intraventricular haemorrhage may occur in 19–33 percent of newborns with SVT (*Wu et al., 2002; Sebire et al., 2005; Fitzgerald et al., 2006*). Therefore, an unexplained intraventricular haemorrhage in a term baby or late-onset intraventricular haemorrhage in a ‘late’ preterm baby should raise the suspicion of SVT (*Ramenghi et al., 2009*).

3. AIMS OF THE STUDY

1. To study the incidence of perinatal and childhood stroke in Estonia (Papers I and II)

2. To study the first clinical signs of perinatal and childhood stroke (Papers I and II)

3. To identify possible risk factors of perinatal and childhood stroke (Papers I and II)

4. To study the outcome of perinatal stroke (Paper I)

5. To study time delay to diagnosis of childhood AIS (unpublished data)

6. To study the association between paediatric ischemic stroke and FVL and PT 20210G>A mutation (Paper III)

4. SUBJECTS AND METHODS

4.1. Inclusion definitions of paediatric stroke

The criteria for identifying ischemic and haemorrhagic stroke were the same in all studies.

The criteria for AIS were clinical symptoms consistent of ischemic stroke (acute focal neurological deficit) with a corresponding focal ischemic lesion on CT or MRI in a known arterial distribution (*Fullerton et al., 2007a*).

The criteria for haemorrhagic strokes were clinical presentation that was consistent with a haemorrhagic stroke (sudden onset of focal neurological deficit, headache, loss of consciousness, or seizure) as well as a CT or MRI showing an IPH or SAH that was consistent with clinical symptoms (*Fullerton et al., 2007b*).

For the diagnosis of cerebral SVT, identification of thrombus within cerebral sinuses and veins by CT venography or magnetic resonance venography was obligatory (*Monagle et al., 2008*).

Transient ischemic attack was defined as focal neurological deficit of acute onset lasting <24 hours with no radiographic evidence of infarct (*Fullerton et al., 2007a*).

Perinatal stroke was divided into two subgroups: (1) perinatal stroke with early diagnosis (acutely diagnosed), defined as systemic or neurologic symptoms within the first 28 days of life with radiographic evidence of an acute or remote vascular event; (2) perinatal stroke with delayed diagnosis (retrospectively diagnosed), defined as no diagnosis of stroke in the neonatal period, clinically congenital hemiparesis or seizures, and radiographic evidence of remote focal infarction, encephalomalacia, and/or porencephaly (*Lynch et al., 2005*). In the present thesis, the term 'neonatal stroke' represents early (or acutely) diagnosed perinatal stroke and the term 'presumed perinatal stroke' is used to represent delayed (or retrospectively) diagnosed perinatal stroke according to recent consensus (*Raju et al., 2007*).

4.2. Subjects

4.2.1. Perinatal stroke (Paper I)

Retrospective (from 1994 to 2002) and prospective (2003) population-based studies were conducted of perinatal stroke in the eastern and southern regions of Estonia (see Figure in Paper I). There were 59,976 live births (30,681 males and 29,295 females) in the study region between 1994 and 2003 (*Estonian Statistics*).

Patients with perinatal stroke were identified using different approaches. Firstly, a list of children from a pilot study was used, based on a search of hospital records from the archives of Estonia's two tertiary children clinics (the Children's Clinic of Tartu University Hospital and Children's Hospital of Tallinn). Searches were conducted for perinatal/neonatal stroke and for hemiparesis. Secondly, new cases of perinatal stroke from January 1 to December 31, 2003 were included in the prospective part of the study. Thirdly, an inquiry (in the form of a letter and telephone calls) was set up among child neurologists and general practitioners in the study region to identify children with congenital hemiparesis. In addition, information was disseminated to doctors through lectures and medical seminars throughout the study period.

The following inclusion criteria were used for patient selection: (1) congenital hemiparesis and/or seizures, (2) ultrasound, CT or MRI performed in the neonatal period revealing an acute or remote ischemic or haemorrhagic (subarachnoid, intraparencymal or intraventricular haemorrhage) stroke, (3) CT or MRI performed after the neonatal period revealing a lesion consistent with remote ischemic or haemorrhagic stroke, (4) gestational age of at least 32 weeks, (5) born between 1994 and 2003, (6) birth registered in one of the eight counties situated in the eastern and southern regions of Estonia (see Figure in Paper I). Exclusion criteria were: (1) other documented diseases with central nervous system (kernicterus, encephalitis, mitochondrial disorders, tumour), and (2) acute stroke after one month of age (childhood stroke).

In total, 53 children (28 boys, 25 girls) with suspected perinatal stroke were identified. At least two investigators critically reviewed the medical records of these children in order to identify the diagnoses of perinatal stroke and, in uncertain cases, one of the reviewers re-investigated the child. The study neuroradiologists reviewed ultrasound, CT and MRI scans. Ultrasound, CT (Siemens AG, Somatom Volume Access or Somatom Spiral HP) and MRI (Siemens AG, Magnetom (Symphony) 1.5 T) were used for the neuroradiological examinations. With the CT, areas of decreased density, cerebral swelling and, in some cases, the haemorrhagic compartment were indicative of an acute stroke. In the late period, it corresponded to areas of hypodensity or porencephaly and brain atrophy in a vascular distribution. With the MRI, the distribution of the signal abnormalities in all patients was identified and classified as cerebral cortex, basal ganglia, internal capsule and periventricular white matter involvement (*Boardman et al., 2005*). Contrast medium was not used, as a rule. In the late period, areas of porencephaly in a vascular distribution lined by white matter, signal abnormalities and volume loss that corresponded to the area of infarction were sought. Local areas of cerebral atrophy and any asymmetries of cerebrospinal fluid spaces were also identified.

Fifteen children were excluded from the study: three extremely premature children (<32 weeks of gestation), four with other diseases of the central nervous system, seven with negative neuroradiology and one child in whom confirmative cranial imaging (MRI or CT) was not performed. The final study

group consisted of 38 children (18 boys, 20 girls), including two retrospectively diagnosed children, who were born in the study area but treated at the Children's Hospital of Tallinn.

EEG, electrocardiography and echocardiography were performed as part of the standard evaluation. Cardiac disorders were investigated in 17/38 cases, including congenital diseases and arrhythmias. Prothrombotic factors were studied in 27/38 cases but only after 2003, meaning that all samples were taken months or years after the acute event. Screening for prothrombotic conditions included total homocysteine, anticardiolipin antibodies, lupus anticoagulant, antithrombin III, protein S, protein C, activated protein C resistance, FVL and PT 20210G>A mutation. The laboratory method was not available for the measurement of lipoprotein (a) levels. Patients' history of ante- and perinatal events was obtained from the obstetrical reports. Infections included congenital infections as well maternal infectious episodes during pregnancy. Maternal diseases included preeclampsia, history of infertility, hyperthyroidism, surgical interventions during pregnancy, abdominal trauma, and systemic illnesses. Factors that implicated placental disorders included clots, placental abruption, chorioamnionitis, fetomaternal transfusion and intrauterine growth retardation. Birth asphyxia was diagnosed by the treating physician.

For neurological outcome analysis, the last visit was documented as being the end of the follow-up period for patients who regularly visited a child neurologist (24 children). A telephone call was made to the family doctors of the remaining children and children were invited to the control visit of two of the study neurologists. Eight children were able to come but the neurological outcome of the remaining six cases was either retrieved from the last visit to a child neurologist or described by the family doctor. All patients had visited a child neurologist at least by the age of two years. No patients died during the follow-up period. In total, the follow-up period lasted an average of seven years (range: 2–12 years).

Hemiparesis was assessed according to the hand function: Stage I – mildly disturbed finger movements, Stage II – significant disturbance of finger movements, Stage III – minimal finger movements, Stage IV – hemiplegia, absence of all movements. Epilepsy was defined as at least two unprovoked epileptic seizures one week after the acute event of stroke.

4.2.2. Childhood stroke (Paper II)

A retrospective and prospective population-based study of childhood stroke was conducted from January 1, 1995 to December 31, 2006. The retrospective part (1995–2003) was carried out at the Children's Clinic of Tartu University Hospital and involved the eastern and southern regions of Estonia (eight counties, Figure in Paper I). The prospective part of the study (2004–2006) involved all of Estonia and was carried out in both of Estonia's tertiary children

hospitals: the Children's Clinic of Tartu University Hospital and Tallinn Children's Hospital. Both parts of the study aimed to identify the clinical symptoms and risk factors of childhood stroke. The incidence rate and case-fatality calculations were based exclusively on the prospective part of the study.

Multiple overlapping data sources were used for complete case ascertainment of patients with childhood cerebrovascular disorders. (1) For the prospective part of the study, child neurologists and physicians from the emergency departments of the two tertiary children's hospitals informed the study team of all new stroke cases. (2) For the retrospective part of the study, patients were identified from a previous pilot study that listed cases of hemiparesis at both tertiary children hospitals. (3) A computerised hospital records database for discharge diagnoses was searched using the *International Classification of Diseases, Ninth Revision* (1995–1996), codes 430–438, 342 and *Tenth Revision* (1997–2006) codes I60-I69, G45 and G81 in both tertiary children hospitals. The same search was performed at the Neurology and Heart Clinics of Tartu University Hospital and in the North Estonia Medical Centre to include adolescents and cardiac surgery patients (up to 18 years of age) treated there. (4) The database of forensic pathologists of the Tartu University Hospital was examined to identify death cases out of hospital. (5) The database of clinical pathologists of the Tartu University Hospital was searched for in-hospital stroke death cases. (6) Patients with focal ischemic brain lesions identified from a population-based study of inflicted traumatic brain injury in Estonia (*Talvik et al., 2006*), with appropriate time criteria, were also included. (7) All paediatricians and family doctors in the study region were informed regarding the start of the prospective study and the information was repeated several times during the study.

Children aged between one month and 18 years with the first-ever cerebrovascular event were included. First-ever stroke in a patient with previous TIAs was registered as an incident of stroke, but a first-ever TIA in a patient with a previous stroke was defined as recurrent (*Fullerton et al., 2007a*).

A review of all data sources identified 59 children aged between one month and 18 years who had a suspected cerebrovascular event. Inpatient and outpatient medical reports were reviewed critically and independently by at least two investigators in order to identify the diagnoses of childhood stroke and uncertain cases were reinvestigated. Paediatric neuroradiology specialists who were blinded to the clinical diagnosis reviewed the scans.

Eleven children (11/59) were excluded from the study: two were excluded because of a confirmed diagnosis of migraine, one of diffuse encephalitis, one of epilepsy and postictal hemiparesis, and one of an arachnoid cyst. Six further patients were excluded due to a lack of imaging confirmation. In two of these six cases, a stroke occurred during the prospective study. An MRI was performed on 4/6 children, including the two from the prospective section of the study. The final study population consisted of 48 children (26 boys, 22 girls) with a confirmed cerebrovascular event. Twenty-four (24/48) children were

diagnosed during the retrospective study (covering the eastern and southern parts of Estonia) and 24/48 during the prospective study (including all of Estonia).

Risk factor analysis included data from case history, cardiological and haematological investigations and brain vessel imaging. The study cardiologist reviewed the electrocardiograms and the echocardiograms. Screening for prothrombotic conditions was performed from May of 2004 and included total homocysteine, anticardiolipin antibodies, lupus anticoagulant, antithrombin III, protein S, protein C, activated protein C resistance, FVL, PT 20210G>A mutation, and C677T and A1298C polymorphisms of the MTHFR gene. Tests with pathological results performed in the acute phase of stroke were repeated at least three months after the stroke onset in order to rule out transient prothrombotic disorders (*Ganesan et al., 1998*). Symptoms and signs of cerebrovascular event were categorised according to the International Paediatric Stroke Study.

4.2.3. Time lag to diagnosis of childhood arterial ischemic stroke

For time lag analysis, children with AIS (n=24) from the epidemiological study (Paper II) were analysed. Two children with an extremely long time delay (one year and three months, respectively) were excluded from the study. Finally, 22 children with AIS were included in the study. The analysis stage involved documenting accurate or estimated time records from the medical charts and radiological database of the following events: (1) first symptom noted by care-givers, (2) first medical examination (ambulance, family doctor or admission to children's hospital), (3) first cranial imaging (mainly CT) and (4) time of diagnosis of AIS, which was the completion time of the neuroimaging test that made the diagnosis.

4.2.4. Case-control study (Paper III)

All children and neonates (n=96) with documented perinatal or childhood ischemic (AIS or SVT) stroke dignosed since 1995 in one of the two tertiary children hospitals in Estonia were potential cases in the case-control study. Because uniform paediatric stroke protocol was not in operation during the early years of the study, a DNA test was performed on 80/96 children. Five children were excluded from the study group: four had major head trauma (two had cerebral contusion and two had birth trauma) and one girl had an iatrogenic stroke due to complicated maxillary hemangioma surgery.

A total of 75 children (45 boys, 30 girls) were included in the study. Nineteen of them had childhood AIS, 49 had perinatal AIS (14/49 had neonatal AIS and 35/49 had presumed perinatal stroke). SVT was diagnosed in 7/75

children (three of whom had neonatal SVT). Additional risk factors were identified in 41 cases (55 percent) (not all were tested for each risk factor). These risk factors were: cardiac pathology (5), protein C deficiency (1), activated protein C resistance without FVL (1), lupus-anticoagulant (1), antithrombin deficiency (1), preeclampsia (8), several miscarriages/stillborns (4), maternal thrombophilia (1), nephrotic syndrome (2), Williams' syndrome (1), meningitis (1), acute gastroenteritis (1), anaemia (1) and hyperhomocysteinemia (6). Forty-eight children (64 percent) were tested for MTHFR gene plymorphisms. MTHFR 677C>T homozygosity was found in four cases (8.3 percent), MTHFR 1298A>C homozygosity in three (6.3 percent) and MTHFR 677C>T/1298A>C double-heterozygosity was detected in eight children (16.7 percent).

The control group consisted of 400 newborns (207 boys, 193 girls) who were born consecutively in all of Estonia's 19 delivery departments in January 2005. The control subjects were included proportionally according to the birth rate in all of Estonia's 16 counties in order to achieve a representative cohort of the nationwide proportion.

For meta-analysis electronic databases (EBSCO, Medline, PubMed, Science Direct, and Scopus) were searched for all case-control studies evaluating FVL and/or PT 20210G>A in children with SVT in November 2009. The search key words were: "FVL or prothrombin mutation" combined with the terms "sinovenous thrombosis, cerebral sinus venous thrombosis, or venous thromboembolism" and "child" or "newborn". The "related articles" option was used and the references of evaluated articles were consulted. Only English language studies were found.

4.3. Data Analysis

4.3.1. Perinatal stroke (Paper I)

Statistical analysis was performed using the SAS Version 8.02 statistical software package (SAS Institute, Cary, NC, USA). Statistical comparisons between normally distributed continuous variables were performed with the Student's T-test. The Kolmogorov-Smirnov criterion was used for the assessment of normality. In order to compare proportions (qualitative variables), the Chi-square test or the Fisher's exact test (when expected values were <5 percent) were used. ORs and 95% CI were used to estimate relative risk. Kaplan-Meier estimation of the proportion of subjects was used during follow up and the log-rank statistic and Cox proportional hazards regression was used to assess differences between the epilepsy-free survival curves. All p values were two-sided and differences were considered statistically significant if the p values were less than 0.05.

4.3.2. Childhood stroke (Paper II)

Incidence rates were calculated as the number of strokes divided by the number of person-years at risk. The mid-year population for children aged between one month and 18 years, obtained from the national statistics bureau, was used in calculations (personal inquiry). Statistical analysis was performed using the SAS Version 8.02 statistical software package (SAS Institute, Cary, NC, USA). Ninety-five percent CIs for incidence rate were calculated based on the Poisson distribution. The two-tailed Fisher's exact test and median two-sample test were used to compare incidence rates and age medians, respectively. All *P* values were two-sided and differences were considered statistically significant if the *P* values were less than 0.05.

4.3.3. Case-control study (Paper III)

Patients' DNA was extracted from peripheral blood using standard methods. For the control group, a robust and efficient method was used to obtain DNA from an anonymous newborn screening specimen. Briefly, DNA was extracted from a 3 mm diameter test card disc, which was soaked in 1 ml distilled water for at least two hours, while vigorously and continuously swirling the samples. The supernatant was discarded, 100µl of methanol was added under ventilation hood and the samples were incubated for 15 min. In the next step, methanol was discarded and 100µl of freshly made 5mM NaOH and 20–50µl of mineral oil was added; the samples were then incubated at 100 for 15 min and placed on ice immediately. The obtained DNA solution can be stored at –20 and easily used in PCR-based applications for several years. FVL and PT 20210G>A polymorphisms were detected simultaneously by duplex polymerase chain reaction and restriction fragment length polymorphism method with *Hind*III, according to the method described by Lucotte et al. (2003) with slight modification. For DNA samples extracted from the test-card specimen, 5µl of DNA solution and 2U Hot Start Taq DNA Polymerase (Fermentas, Vilnius, Lithuania) was used in PCR reaction in a volume of 50µl and the number of PCR cycles was increased from 37 to 42 cycles.

Statistical analysis was performed using the SAS Version 8.02 statistical software package (SAS Institute, Cary, NC, USA). A comparison between the study and control group was performed with a chi-square test and Fisher's exact test when the expected values were <5 percent. Results are given as OR with 95% CI.

For meta-analyses, comprehensive Meta Analysis Version 2 (Biostat, Englewood, NJ, USA) was used to pool data from the case-control studies. Comparisons were performed for FVL or PT 20210G>A carriers (heterozygous or homozygous) versus non-carriers. Data from the present study was included. Weighted ORs with 95% CIs were calculated by using fixed-effects models and

a chi-square test was performed to test for heterogeneity. The Egger test was performed to check for publication bias.

4.4. Ethical approval

The study was approved by the Ethics Review Committee on Human Research of the University of Tartu. Ethical permission to review medical charts of the retrospective study and informed consent was obtained from the parents and children for participation in the study in the prospective part of the study in Studies I and II. For Study III, ethical permission to perform anonymous DNA testing from control patients and informed consent was obtained from the patients and/or their parents to perform DNA analysis.

5. RESULTS

5.1. Incidence of paediatric stroke in Estonia

5.1.1. Incidence of perinatal stroke

The diagnosis of perinatal stroke was confirmed in 38 children (18 boys, 20 girls). Twelve of the 38 children (nine boys, three girls) had neonatal stroke, while the remaining cases (nine boys, 17 girls) had presumed perinatal stroke. With 59,976 live births in Estonia over the 10-year study period, the incidence rate of perinatal stroke in the country is 63.4/100,000 or one for every 1578 live births (95% CI: 46.17 to 86.95). The corresponding figure is 58.7/100,000 for boys and 68.3/100,000 for girls.

The incidence rate of perinatal stroke recognised in the newborn period (neonatal stroke) is 20.0/100,000 or one per 4998 live births (29.3/100,000 for boys and 10.2/100,000 for girls). The incidence rate of presumed perinatal stroke is 43.4/100,000 or one per 2307 live births (29.3/100,000 for boys and 58.0/100,000 for girls).

5.1.2. Neuroradiological features of perinatal stroke

Cranial radiology scans (ultrasound, MRI or CT) were performed in all children with neonatal stroke (12/38). Acute arterial ischemic lesions were identified in 4/12 neonates in the initial images during the neonatal period – all infarcts were in the region of the left middle cerebral artery. Haemorrhagic lesions were diagnosed in the other 7/12 children: SAH with periventricular oedema in one, parenchymal bleeding in two and intraventricular haemorrhage in four children. Intraventricular haemorrhage was unilateral in three of the four cases. In one case (1/12), MRI on the eighth day of life revealed asymmetrical porencephalic dilatation of the lateral ventricles and hemiatrophy referring to antenatal onset of the vascular event. After the neonatal period, neuroimaging demonstrated a large porencephalic lesion because of the arterial ischemic and/or haemorrhagic insult as the main finding in 11 of 12 cases and small unilateral periventricular cysts in one case.

In children with presumed perinatal stroke, neuroimaging (CT or MRI) was performed at a mean age of 25 months (range: one month to five years). Eleven of the 26 children had cortical damage, in 4/26 children basal ganglia or internal capsule were affected without cortical damage, and lesions of the periventricular white matter alone were present in 11/26 cases. Porencephaly was described in 13 cases and T2-hyperintensities in another seven cases. Four children had asymmetrical dilatation of lateral ventricles with signal change of

periventricular white matter, one had cortical focal atrophy and one had bilateral central and peripheral atrophy as the main finding.

Altogether, unilateral left-sided brain damage was seen in 20/38 cases and unilateral right-sided in 4/38 cases. In the remaining cases, brain damage was bilateral but asymmetrical with predominating left side involvement in 11/38 and predominating right side involvement in 2/38 cases. In one case, the lesion was bilateral symmetrical but clinically the child had stage III right-sided hemiparesis.

5.1.3. Incidence of childhood stroke

The prospective study included 805,244 person-years of observation (413,569 males and 391,676 females) over the three-year study period. At the same time, a total of 24 cases (14 boys, 10 girls) of first-ever cerebrovascular event were confirmed, yielding an overall annual incidence rate of 2.98 per 100,000 person-years. The diagnosis of TIA was confirmed in three children (two boys, one girl). One of them had an ischemic stroke three weeks after the TIA episode and was included in the AIS incidence rate calculations.

Stroke (excluding TIA without following arterial ischemic stroke) was diagnosed in 22 children (13 boys, nine girls), which yielded an annual incidence rate of 2.73 per 100,000 person-years. Table 3 shows the incidence rates of stroke subtypes with gender disparities. AIS was diagnosed in 13/22 cases (59 percent), IPH in 7/22 cases (32 percent) and SVT in 2/22 cases. There were no significant differences between gender distribution and stroke incidence rates (P=0.6). No SAH cases were identified during the prospective study.

Table 3. Incidence rates for stroke subtypes

Type of	***Total***		***Boys***		***Girls***		
stroke	***Cases***	***IR (95% CI)***	***Cases***	***IR (95% CI)***	***Cases***	***IR (95% CI)***	***P-value***
AIS	13	1.61 (0.74–2.49)	9	2.18 (1.14–4.14)	4	1.02 (0.40–2.63)	0.312
IPH*	7	0.87 (0.23–1.51)	3	0.73 (0.25–2.13)	4	1.02 (0.40–2.63)	0.941
SVT	2	0.25 (0.00–0.59)	1	0.24 (0.04–1.37)	1	0.26 (0.05–1.45)	1.000
Stroke	**22**	**2.73 (1.91–4.43)**	**13**	**3.14 (1.84–5.38)**	**9**	**2.30 (1.21–4.37)**	**0.611**

* No cases of SAH were identified in the prospective section.

The median age of symptom onset in the total group was 10.1 years (interquartile range: 4.4–15.3 years). It was 6.4 years for children with AIS (interquartile range: 2.1–10.2) and 12.2 years for children with IPH (interquartile range: 6.7–17.2, *P*=0.17). The incidence rate of all events, as well as the incidence rate of IPH, was higher among adolescents than it was for younger children, who had more frequently ischemic events (see Figure 1 in Paper II).

5.1.4. Thirty-day case-fatality of childhood stroke

Three children (3/22) died within 30 days of a stroke, yielding an overall 30-day case-fatality rate of childhood stroke of 14 percent. As all the three children had IPH (one of them had intracerebellar haemorrhage), the 30-day case-fatality rate for IPH was 43 percent (3/7). All deaths occurred within two to four days of stroke onset. The etiological factors of these cases were aneurysm, arterio-venous malformation and lupus nephritis with antiphospholipid syndrome. No patients with AIS died during the first 30 days.

5.2. Clinical presentation of paediatric stroke

5.2.1. Clinical presentation of perinatal stroke

All children with neonatal stroke (12/38) presented symptoms within the first days of life (see Table 4). The most frequently registered symptoms were seizures (11/12), changes in muscular tone (9/12) and disturbed level of alertness (8/12). In patients with neonatal seizures, epileptic dischargers on EEG were confirmed in 3/11 cases. Hypoxic-ischemic encephalopathy (according to Sarnat and Sarnat, 1979) was diagnosed in 10/12 newborns: grade I in one, grade II in 7 and grade III in 2 cases. Nine neonates required intensive care treatment and five were intubated and needed artificial ventilation.

Among the 26 presumed perinatal stroke cases, only six (23 percent) presented mild clinical signs in the neonatal period (Table 4). Mild changes of muscular tone were documented in five of these cases. After the neonatal period, 22/26 children required medical attention because of hemiparesis and 4/26 due to seizures. The mean age at the emergence of symptoms was 8.0 months (range: one month to two years).

Table 4. Clinical presentation of perinatal stroke in the neonatal period

Syptoms	***Neonatal stroke*** **N = 12 (%)**	***Presumed perinatal stroke*** **N = 26 (%)**
Seizures	11 (92%)	–
Focal	5	–
Secondarily generalized	6	–
Muscular tone changes	9 (75%)	5 (19%)
Hemiparesis	4	2
Hypotony	4	2
Hypertony	1	1
Disturbed level of alertness	8 (67%)	2 (8%)
Irritability	4	2
Lethargy	4	–
Respiratory problems	7 (58%)	2 (8%)
Feeding difficulties	1 (7%)	1 (4%)

5.2.2. Age at onset and clinical presentation of childhood stroke

The median and mean ages at symptom onset for children from both the retrospective study and the prospective study were 11.4 and 10.4 years for TIA, 5.9 and 6.4 years for AIS, 10.0 and 8.9 years for IPH, 15.0 and 15.0 years for SAH, and 15.5 and 15.5 years for SVT patients.

Table 5 lists the main clinical symptoms of the patients from retrospective and prospective studies. As the table shows, diffuse signs (headache and decreased level of consciousness) were more common in patients with IPH and SVT, compared to AIS (93–100 percent vs. 42 percent respectively), whereas focal symptoms were seen more often in patients with AIS (100 percent vs. 64 percent, respectively). In patients with AIS, hemiparesis was right-sided in 16/22 cases (73 percent) and left-sided in 8/22 cases (36 percent). Seizures were present in three patients (3/24, 13 percent) with AIS and in 5/14 patients (36 percent) with IPH. Children with AIS aged between one month and one year (3/24) did not present seizures more often than older children (1/3 vs. 2/21, *P=0.34)* or altered mental status (1/3 vs. 7/21, *P=1*). Similarly, children with IPH aged <1 year (3/14) did not present with seizures more often than older children (2/3 vs. 3/11, *P=0.51*) or altered mental status (2/3 vs. 11/11, *P=0.21*).

Table 5. Clinical presentation of childhood stroke and TIA in the acute period

Symptom	*TIA N=6 (%)*	*AIS N=24 (%)*	*ICH N=14 (%)*	*SAH N=2 (%)*	*SVT N=2 (%)*
Focal signs	**6 (100)**	**24 (100)**	**9 (64)**	**–**	**1 (50)**
Hemiparesis	6 (100)	22 (92)	9 (64)	–	–
Visual field deficit	–	2/21* (10)	–	–	–
Speech deficit	3 (50)	8/21* (38)	2/11* (18)	–	1 (50)
Ataxia	–	4 (17)	–	–	–
Diffuse signs	**–**	**10 (42)**	**13 (93)**	**2 (100)**	**2 (100)**
Decreased level of consciousness	–	8 (33)	13 (93)	1 (50)	1 (50)
Headache	–	5/21* (24)	8/11* (73)	2 (100)	2 (100)
Seizures	**–**	**3 (13)**	**5 (36)**	**–**	**1 (50)**
Other signs	**–**	**9 (38)**	**7 (50)**	**1 (50)**	**2 (100)**
Vomiting	–	6 (25)	6 (43)	1 (50)	2 (100)
Fever	–	5 (21)	3 (21)	1 (50)	–

* Visual field defect, aphasia and headache were not assessable in infants.

5.3. Risk factors of paediatric stroke

5.3.1. Risk factors of perinatal stroke

Previously identified risk factors for perinatal stroke (up to 2006) were identified in 12 cases (32 percent). These were made up of: Protein C deficiency (2), hyperhomocysteinemia (3), preeclampsia (7), intrauterine growth restriction (2), and chorionamnionitis (3). Four children had more than one risk factor. Four new studies have been published since 2007 that have assessed the maternal and peripartum risk factors (*Benders et al., 2007; Chabrier et al., 2010*) or prothrombotic risk factors (*Herak et al., 2009; Simchen et al., 2009*) of perinatal AIS. In the view of pooled data from earlier and recent case-control studies, risk factors were present in 87 percent (33/38) of children with perinatal stroke. Additionally, primiparity (19), emergency caesarean section (8), Apgar score <7 at one minute (8), cord pathology (6), rescusitation at birth (4), history of foetal loss (4), intrauterine growth restriction (2), prolonged second stage of the delivery (2), vacuum delivery (1), PT 20210G>A mutation (1), and antiphospholipid antibodies (1) were identified. All five cases that did not have risk factors had presumed perinatal stroke with uneventful pregnancies. In two of these five cases, delivery and thrombophilia screening took place without any pathology, but they had dyslipoproteinemia in blood analyses. Another two children were delivered via elective surgery and in one case, the delivery was rapid (<5 hours). Thrombophilia screening was performed partly to these three children.

12

A comparison between children with neonatal stroke and presumed perinatal stroke revealed male predominance within children with early diagnosis. Statistically, children with neonatal stroke were born more often from the first delivery and had greater birth weight ($P<0.05$, see Table 3 in Paper I). Placental pathology and asphyxia occurred significantly more often among children with presumed perinatal stroke ($P<0.01$).

5.3.2. Risk factors and neuroimaging features of childhood stroke

Thirty-one percent of patients (15/48) had a previously known underlying condition; 5/6 (83 percent) children with TIA, 15/24 (63 percent) children with AIS, 12/16 (75 percent) children with haemorrhagic stroke and one of two children with SVT were considered healthy prior to the stroke.

Transient ischemic attack. Six children with TIA and without AIS during follow-up had the following risk factors: one (1/6) had a history of acute lymphoid leukaemia. Thrombophilia screening was performed on 4/6 children and hyperhomocysteinemia was detected in three children. Cardiac investigations were performed on 3/6 children, and ventricular septal defect was detected in one child. One patient (1/6) was not investigated for risk factors. Of the three patients with TIA followed by AIS, one had patent *foramen ovale*, but the other two patients were not investigated thoroughly.

Arterial ischemic stroke. MRI was performed in 21/24 patients and CT only in 3/24 children with arterial ischemic stroke. Diffusion-weighted MRI was performed in the acute phase in 5/24 children. Ischemic lesions were located in the left hemisphere in 12/24 (50 percent), in the right hemisphere in 8/24 (33 percent) and bilaterally in 4/24 (17 percent) cases. In 15/22 AIS cases (68 percent), the lesion was located in the basal ganglia and/or internal capsule, whereas in 7/22 cases (32 percent) it was located in the cerebral lobes.

In order to study the risk factors, cardiac investigations (electro- and echocardiography) were performed in 18/24 children with arterial ischemic stroke, thrombophilia screening in 18/24, and cerebral vascular imaging (MRA) in 16/24. In 5/18 investigated cases (24 percent), a possible intra- or transcardiac reason was identified (patent *foramen ovale* in four cases and sinus arrhythmia in one case). Inherited and acquired hypercoagulable situations were present in 9/24 children: prothrombotic factor (seven of 18 investigated cases, 39 percent), nephrotic syndrome in one child and infection-related dehydration (possible mechanism) in three children. Among prothrombothic factors, six children had hyperhomocysteinemia and two children had TT677 genotype of MTHFR gene, a combination of them was present in one child.

MRA revealed arteriopathy in 4/16 children (25 percent). Of these, unilateral stenosis (2) or occlusion (1) of the proximal part of middle cerebral artery occurred in three patients and bilateral occlusion of anterior and middle cerebral arteries and unilateral stenosis of posterior cerebral artery was found in one child with William's syndrome. Anatomical (rather than pathological) aberrations of cerebral vessels were described in another four of the 16 (25 percent) cases. Head trauma was in anamnesis in 7/24 children, comprised of inflicted brain injury (2), cerebral contusion (1) and mild head trauma (4). In one case, AIS was a complication of maxillary hemangioma surgery and brain ischemia occurred in one case as a complication of bacterial meningitis.

Haemorrhagic stroke. Haemorrhagic stroke was diagnosed in 16 patients. IPH was detected in 14 of these cases and SAH in the other two. Arteriovenous malformation was identified in 7/14 cases of IPH; other causes were aneurysm (1), use of amphetamine (1), hypertension caused by glomerulonephritis (1), lupusnephritis with antiphospholipid syndrome and peritoneal dialysis (1), and meningoencephalitis (1). The cause of IPH remained unknown in 3/14 cases. Subarachnoid haemorrhage was caused by basilar artery aneurysm in one case; the cause of the other case remained unknown but haemorrhage was preceded by extensive psychical and physical stress.

Sinovenous thrombosis. Two cases of SVT were diagnosed and possible aetiology was identified in both. The first child had hormone-dependent minimal change nephrotic syndrome since two years of age as an underlying disease and developed SVT with multiple locations (superior sagittal sinus, venous confluence, straight sinus, left transverse sinus, sigmoid sinus, and rear part of internal jugular vein) but without infarction after an exacerbation of the disease and excessive diuretic treatment at 15 years of age. The other patient was a heterozygous carrier of both FVL and PT 20210G>A mutation and homozygous to MTHFR 1298A>C polymorphism and had left temporal haemorrhagic infarction concomitantly with transverse and sigmoid sinus thrombosis.

In total, risk factors were identified in 41/48 of children with with cerebrovascular disease (85 percent). In 21/24 AIS cases (88 percent), at least one risk factor was identified, with more than one risk factor identified in 6/24 (25 percent) children. Eight of 15 children with one identified risk factor were only partly investigated.

5.4. Outcome of perinatal stroke

All children with perinatal stroke had evidence of hemiparesis at outcome. It was right-sided in 32/38 cases (84 percent) and left-sided in 6/38 (16 percent), grade I (mild) in nine cases, grade II (moderate) in 17 cases and grade III (severe) in 12 children. Severe hemiparesis (stage III) occurred more often

among patients with neonatal stroke (OR=6.9; 95 percentCI 1.40; 33.57, Table 6).

Thirteen children in the study group (34 percent) had developed epilepsy by the mean age of three years (range: nine months to seven years). Six children had complex focal seizures, five had simple focal seizures and two had complex focal seizures with secondary generalisation. Epilepsy occurred in the same proportion of children with neonatal stroke and presumed perinatal stroke (log-rank *P*=0.916, Table 6).

Table 6. Outcome of perinatal stroke

	Neonatal stroke N=12	***PPS N=26***	***OR***	***95% CI***	***P value***
Grade of hemiparesis					
Severe	7 (58%)	5 (19%)	5.9	1.30; 26.5	***P*=0.0258**
Mild-moderate	5 (42%)	21 (81%)			
Epilepsy	4 (33%)	9 (35%)	1.1*	0.30; 3.39	*P*=0.9820

PPS – presumed perinatal stroke; * Relative risk (RR)

5.5. Time lag to diagnosis of childhood arterial ischemic stroke

Figure 2 shows the median time delays. AIS developed in previously sick children in the hospital in three of the 24 cases. The total median delay (interval from symptom onset to diagnosis of AIS) was 132 hours (5.5 days, at average 9.2 days). There was no significant difference between retrospective and prospective studies (*P*=0.745). Within the first six hours after symptom onset, initial medical assessment was performed in 64 percent of the children (14/22) and first neuroimaging in 50 percent (11/22). The diagnosis of AIS was established within six hours after symptom onset in 18 percent of the children (4/22).

Only nine of 22 initial neuroimaging scans (CT in 20 cases) were considered indicative of ischemic stroke (see Figure 3). The misinterpretation was caused by normal findings in 6/22 cases and by misleading features on neuroimaging in 7/22 cases. Altogether, the diagnosis of AIS was the first choice of medical personnel in 12/24 cases (50 percent). In the remaining cases, the following initial diagnosis was suspected: encephalitis (1), meningitis (1), brain tumour (3), cerebral commotion (3), cerebral contusion (2), epileptic postictal hemiplegia (2). The final diagnosis was made by a confirmative MRI/MRA study with an average delay of 2.4 months (range: two days to 13 months).

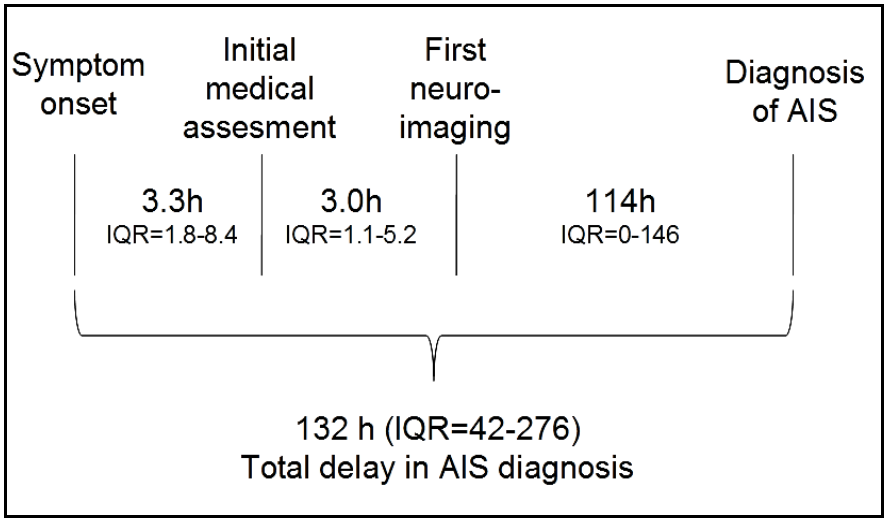

Figure 2. Median time delays in diagnosis of childhood AIS

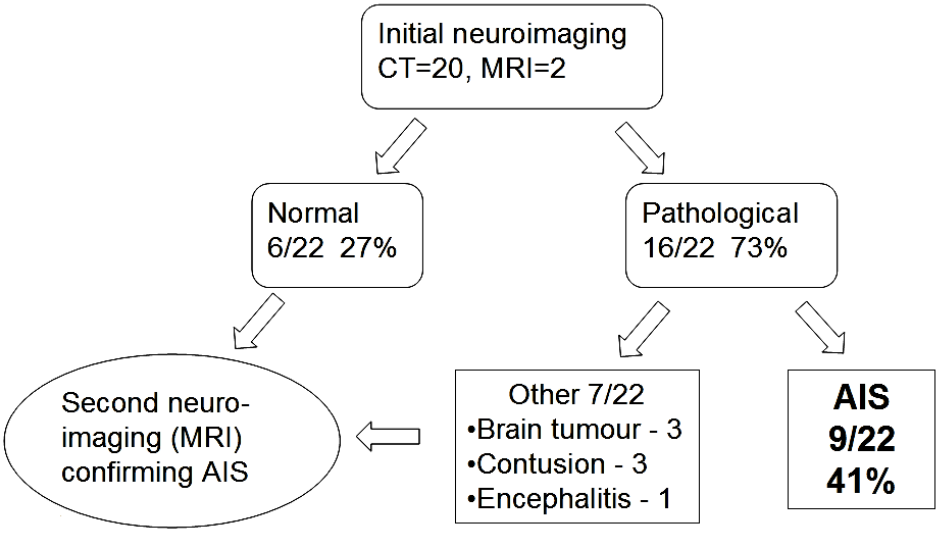

Figure 3. Neuroimaging features in 22 children with childhood AIS

5.6. Association between factor V Leiden and prothrombin 20210G>A mutation and paediatric ischemic stroke

5.6.1. Case-control study

FVL heterozygosity was found in three patients: one with perinatal AIS (presumed perinatal stroke), one with neonatal SVT, and one with childhood SVT. PT 20210G>A heterozygosity was also detected in three children, one of whom had perinatal AIS (presumed perinatal stroke) and two of whom had childhood SVT. No homozygote was identified in the study group.

A 16-year-old girl with SVT was heterozygous for both FVL and PT 20210G>A mutation. She was also homozygous to MTHFR gene 1298A>C polymorphism. One year after the SVT event, she gave birth to a son. During pregnancy, she received low molecular weight heparin treatment and both pregnancy and delivery were uncomplicated. In family history, many relatives had suffered from cerebrovascular accident in their sixties. In relation to this patient's gene findings, other relatives were tested. Her father was not a carrier of FVL nor PT 20210G>A mutation but he was double heterozygous for MTHFR 677C>T and 1298A>C polymorphisms. The patient's mother was not investigated for gene mutations but she should be a carrier of FVL and PT 20210G>A mutation, as a cousin from her mother's side was tested during pregnancy and she was double-heterozygote for FVL and PT 20210G>A mutation (she was also treated with low molecular weight heparin during pregnancy.)

The population recorded a heterozygotes prevalence of three percent for the FVL (12/400) and 3.25 percent for PT 20210G>A mutation (13/400). As no homozygotes for either mutations were recorded, allele prevalences for the FVL and PT 20210G>A mutation were 1.5 percent (1:67) and 1.6 percent (1:61), respectively. The frequencies (patients vs. controls) and ORs with 95% CIs obtained from the comparison between children with ischemic stroke and the control group are summarised in Table 7.

Both FVL and PT 20210G>A occurred statistically significantly more frequently among patients with SVT than in the control population (OR=12.9, 95% CI: 2.29–73.0; OR=11.9, 95% CI: 2.11–67.2, respectively). The difference between childhood/perinatal AIS and the controls was not significant.

Table 7. Prevalence of factor V Leiden and prothrombin 20210G>A mutation in cases and controls

	Cases			*Controls*				
Type of stroke	***Het***	***Total***	***%***	***Het***	***Total***	***%***	***OR***	***95% CI***
Factor V Leiden	**3**	**75**	**4.0**	**12**	**400**	**3.0**	**1.35**	**0.37–4.90**
Childhood AIS	0	19	0.0				–	
Perinatal AIS	1	49	2.1				0.67	0.09–5.30
SVT	2	7	28.6				12.9	2.28–73.0
PT 20210G>A	**3**	**75**	**4.0**	**13**	**400**	**3.25**	**1.24**	**0.34–4.46**
Childhood AIS	0	19	0.0				–	
Perinatal AIS	1	49	2.1				0.62	0.08–4.85
SVT	2	7	28.6				11.9	2.11–67.2

Het = heterozygotes

5.6.2. Meta-analyses of factor V Leiden and prothrombin 20210G>A mutation in paediatric sinovenous thrombosis patients

Six case-control studies from five countries, including the present study, with 258 SVT case subjects and 1265 control subjects, were included in the meta-analysis on FVL (*Hagstrom et al., 1998; Bonduel et al., 2003; Heller et al., 2003; Kenet et al., 2004; Miller et al., 2006*). The prevalence of FVL among case subjects was 12.8 percent and 3.6 percent among control subjects. Carriers of the FVL were 3.1 times more likely to develop SVT (95% CI: 1.8–5.5), see Figure 1 in Paper III. No significant inter-study heterogeneity was observed (P=0.66). The Egger test result was not significant (P=0.96), which suggests a low probability of publication bias. Meta-analyses performed separately for neonatal and childhood SVT showed a significant association between FVL mutation and neonatal SVT (OR=5.5, 95% CI 2.1–14.5) but no significant association with childhood SVT (see Table 2 in Paper III).

Five case-control studies from five countries, including the present study, with 249 SVT case subjects and 1200 control subjects, were included in the meta-analysis on PT 20210G>A (*Bonduel et al., 2003; Heller et al., 2003; Kenet et al., 2004; Miller et al., 2006*). PT 20210G>A was present in 5.2 percent of case subjects and 2.5 percent of control subjects. Carriers of the FVL were 3.1 times more likely to develop SVT (95% CI: 1.4–6.8) (see Figure 2 in Paper III). No significant inter-study heterogeneity was observed (P=0.45). The Egger test result was not significant (P=0.81), which suggests a low probability of publication bias. Meta-analyses performed separately for neonatal and childhood SVT showed a significant association between PT 20210G>A mutation and childhood SVT (OR=5.3, 95% CI 1.4–19.8) but no significant association with neonatal SVT (see Table 2 in Paper III).

6. DISCUSSION

Every country requires epidemiological studies in order to obtain evidence-based data. This data is extremely important for planning medical and social care. On one hand, these studies provide more accurate data regarding the profile of the subtypes of disease, clinical symptoms, age variations, gender distributions, etiological factors, diagnostic strategies and outcome results than do reports about hospital case series. On the other hand, the quality of these studies depends on the quality of diagnosis, the size of study chohort and the technical and laboratory equiqment used.

Estonia, with its small population, well-developed medical care, solidarity-based insurance system and strong cooperation between different levels of medical specialists (family physicians, paediatricians and neurologists) and researchers of Tartu University, facilitates epidemiological studies in the field of paediatric neurology.

6.1. Incidence of paediatric stroke in Estonia

6.1.1. Incidence and neuroradiological features of perinatal stroke

According to this study, the incidence rate of perinatal stroke in Estonia is 63.4 per 100,000 live births. As far as can be ascertained, this is the third published study that has aimed to determine the incidence rate for both neonatal and presumed perinatal stroke. Two previous population-based studies of perinatal stroke considering both neonatal and presumed perinatal stroke found incidence rates lower than the present study (*Wu et al., 2004; Lee et al., 2005*; Table 1). One reason for this could be that both studies only included arterial ischemic strokes, whereas the present study also included children with haemorrhagic stroke, as well as children with AIS presented as unilateral periventricular white matter lesions, as classified above (*Boardmann et al., 2005; Wu et al., 2006*).

In order to make a comparison with prior studies of neonatal stroke, it was necessary to separately analyse the incidence of perinatal arterial stroke diagnosed in the neonatal period (20.0 per 100,000 live births), which is lower than in earlier studies (Table 1), even though haemorrhagic cases were included. There is no current consensus regarding whether to include only ischemic cases or to also include haemorrhagic cases in epidemiological studies of perinatal stroke. It was decided to include haemorrhagic cases of perinatal stroke in this study because the pathogenetic mechanisms are often combined in perinatal damage: the neuroradiological follow-up showed porencephaly in arterial distribution in later stage in six of the seven cases with intracranial bleeding diagnosed in the neonatal period.

Eleven of the 12 neonates with early diagnosis demonstrated evidence of an acute brain insult in neuroradiology. This data correlates well with the study of Cowan et al. (2003), which showed that 80 percent of term newborns with encephalopathy had evidence of acute hypoxic-ischemic brain lesions in MRI. That finding contradicted the previous opinion about the brain damage during the foetal period. In the present study, hypoxic-ischemic encephalopathy was diagnosed in 83 percent of cases of neonatal stroke. This supports the need for MRI investigation in all children with clinical symptoms of encephalopathy. Left-sided brain damage occurred more often than right-sided in the study group, which is consistent with other results (*Ramaswamy et al., 2004; Golomb et al., 2001; Schulzke et al., 2005; Lee et al., 2005; deVries et al., 1995*). In one-third of cases, the other side of brain was also affected, albeit to a smaller extent. Studies on animal models of neonatal stroke have shown that after a large unilateral neonatal hypoxic brain injury, apoptotic processes are present in both hemispheres (*D'Arceuil et al., 2000*). No difference in side of brain damage was observed between children with neonatal stroke and presumed perinatal stroke.

A subject of debate has been which remote brain lesions on neuroimaging should be classified under presumed perinatal stroke in children with congenital hemiparesis. An excellent review of this topic was published recently (*Kirton et al., 2008*), which defines the vascular syndromes of presumed perinatal stroke. According to that study, brain lesions of presumed perinatal stroke can be divided into the following categories: (1) cortical, caused by the interruption of blood flow in the main braches of the cerebral arteries, and (2) subcortical, caused by either (a) AIS in the territory of lateral lenticulostriate arteries or (b) infarction in venous territory. Lateral lenticulostriate stroke is characterised by lesions in basal ganglia and the posterior limb of the internal capsule, while periventricular white matter encephalomalacia with relatively spared basal ganglia is characteristic of remote periventricular venous infarction (*Kirton et al., 2008*). In that study's cohort, main branches of middle cerebral artery (cortical damage) were affected in 66 percent of the cases, lateral lenti-culostriate stroke occurred in seven percent of cases and periventricular venous infarction in 20 percent of children with presumed perinatal stroke. Rare lesions occurred in seven percent of cases. The present study showed a greater proportion of subcortical damage: neuroimaging revealed cortical damage in 42 percent of children with presumed perinatal stroke, probable lateral lenti-culostriate stroke in 15 percent and probable periventricular venous infarction in 42 percent of children.

It is difficult to estimate the exact timing of brain lesions in children with presumed perinatal stroke when the first neuroradiological study is performed several months after the acute event. A vascular event may have occurred before labour, during labour or (subclinically) in the neonatal period (*Govaert et al., 2009a*). In some cases, neuroimaging provide indisputable evidence; for example, porencephaly with polymicrogyria can refer to an antenatal onset in an

14

infant born after 26 weeks' of gestation (*Govaert et al., 2009a*). Further investigations are needed to specify whether perinatal stroke with delayed clinical presentation takes place rather before, during or after the delivery.

According to definition, neonatal haemorrhagic stroke includes IPH and SAH (*Govaert et al., 2009a*). In the present study, haemorrhagic lesions were found in seven of 12 children with neonatal stroke, including four children with intraventricular haemorrhage. In all cases of intraventricular haemorrhage, only ultrasound was performed in the neonatal period; after the neonatal period, neuroimaging demonstrated large porencephalic lesions in periventricular or cortical regions, which implies that cerebral parenchyma was involved in the cerebrovascular event. The possibility that not all haemorrhagic cases had arterial origin cannot be also excluded.

6.1.2. Incidence and 30-day case-fatality of childhood stroke

The nationwide prospective population-based study identified the incidence rate of childhood stroke in Estonia as 2.73 per 100,000 person-years for children aged between one month and 18 years. Including patients with TIA, the incidence rate is 2.98 per 100,000 person-years. The study data correlate well with previous population-based studies (Table 2). The incidence rate of childhood stroke in Estonia is markedly lower than perinatal (63/100,000) or adult stroke rates (233/100,000, *Vibo, 2007*) in Estonia. The study revealed an incidence rate of AIS 1.61 per 100,000 person-years, which is similar to previous data of 0.6–2.1/100,000 (Table 2); this assumes the exclusion of the study from France with an extremely high incidence rate (7.9/100,000). An increase of childhood stroke incidence would have been expected in more recent studies as a consequence of the increased recognition and possibilities of modern neuroradiology (*Pappachan and Kirkham, 2008*). Kleindorfer et al. (2006) studied temporal trends in the incidence of stroke in children and did not find a statistically significant increase over a 10-year period. However, there was a trend towards an increase in the incidence of strokes over time and a larger sample size may have demonstrated the difference.

The current study suggests an incidence rate of childhood SVT (excluding neonates) of 0.25/100,000 person-years. Previous studies have found the incidence rate of childhood SVT to be 0.35 per 100,000 (*Heller et al., 2003*) and 0.38/100,000 (*deVeber et al., 2001*), which are slightly higher than the present study. If there had been one more patient with SVT diagnosis during the epidemiological study period, the incidence rate of childhood SVT would have been 0.37 per 100,000, which is very similar to the results of Heller and deVeber. Admittedly, one patient could have been underdiagnosed during the study period. Many authors feel that SVT is underdiagnosed because its symptoms are non-specific and the diagnosis can be missed if CT venography or magnetic resonance venography is not performed (*Sebire et al., 2005*).

TIA is not routinely included in childhood stroke studies, most probably because the symptoms only last for a short time and children do not reach medical attention or the symptoms are misinterpreted. This study is the first to suggest a childhood TIA incidence rate of 0.37 per 100,000 person-years. Three patients were found to have TIA during the study period, which represented 14 percent (3/22) of the total group of childhood cerebrovascular disorders. This is similar to the proportion (12.5 percent) in the only population-based study on childhood stroke that included TIA (*Zahuranec et al., 2005*). There is a possibility that some patients who suffered from TIA with serious comorbidities may not have been coded by their treating physicians (personal contact).

In the current study, all stroke cases were investigated in one of two tertiary children's hospitals of Estonia. Using a multitier process for case ascertainment, Fullerton et al. (2007b) found that nine percent of haemorrhagic stroke cases had only outpatient diagnostic evaluation. In Estonia, outpatient diagnostic evaluation of childhood stroke is excluded by a consensus agreement that all children with any neurological or developmental problems should be consulted and investigated by a child neurologist in one of the two tertiary children's hospitals. A well-developed health care system and solidarity insurance system would be likely to exclude the possibility of losing study patients because of the excessive distances or prohibitively expensive medical aid involved (*Sudlow and Warlow, 1996*).

Sixty-seven percent of the patients of ischemic stroke in the prospective study group were males (male:female ratio: 1.4:1). Despite this, the study did not show a significant ($P>0.05$) male predominance, probably because of the small sample size. Zahuranec et al. (2005) showed that a childhood population of five million is needed in order to prove a 1.3:1 rate ratio in paediatric stroke studies. However, the result of the current study is in concordance with previous findings, including that of the International Pediatric Stroke Study of 1187 children, which indicated a male-to-female ratio of 1.5:1 (*Golomb et al., 2009*).

According to previous studies, half of stroke cases occur before the age of six years (*Lanthier et al., 2000*) and infants have the highest annual stroke incidence of any age group (*Fullerton et al., 2003*). In contrast, the median age of stroke onset in the current study was 10.1 years. As found in previous studies (*Fullerton et al., 2003*), AIS was more commonly diagnosed in younger children and haemorrhagic stroke in older children (see Figure 1 in Paper II).

The prospective study did not reveal any death cases of childhood AIS within 30 days. Previous studies have found 30-day case-fatality among childhood AIS patients to be 4–16 percent (*Lanthier et al., 2000; Broderick et al., 1993; Fullerton et al., 2003; Fullerton et al., 2007a*). Some cases may be missed as forensic and in-hospital autopsy databases were searched only in Tartu. On the othe hand, our data support the finding of Fullerton et al. (2002) that mortality from strokes in children has been decreasing over the years. At the same time, three out of seven haemorrhagic stroke cases in the present study died, which yielded a 30-day fatality rate of 43 percent. This is slightly higher

than the 20–34 percent that has been reported previously (*Broderick et al., 1993; Lanthier et al., 2000; Fullerton et al., 2007a; Lo et al., 2008*).

6.2. Clinical presentation of paediatric stroke

6.2.1. Clinical presentation of perinatal stroke

Given that the diagnosis of perinatal stroke is delayed in two-thirds of cases, the study was interested to determine the feasibility of promoting diagnosis earlier. The study found that neonatal seizures were the most common clinical finding that leads to further investigations, which correlates with previous studies (*Fujimoto et al., 1992; Jan and Camfield, 1998; Sreenan et al., 2000; Wu et al., 2004; Golomb et al., 2008b; Chabrier et al., 2010*). Neonatal seizures were not mentioned in the medical records in any case of presumed perinatal stroke, which implies that in order to improve the early diagnostics of perinatal stroke it is necessary to look carefully for clinical signs other than neonatal seizures. One in five of the retrospectively diagnosed children presented with changes of muscular tone during the neonatal period. These changes should serve as indications of imaging.

6.2.2. Clinical presentation of childhood stroke

Most of the cases of childhood AIS (92 percent) presented with hemiparesis in the present study. Seizures were present in 13 percent cases of AIS, which is a smaller proportion than the 18–35 percent reported in earlier studies (*Eeg-Olofsson et al., 1983; Zahuranec et al., 2005; Shi et al., 2008; Giroud et al., 1997*). Zimmer et al. (2007) proposed that children with cerebral ischemic stroke aged under one year were more likely to present with epileptic seizures and altered mental status than children aged over one year. The present study was unable to confirm this association as there were only a few children aged less than one year in the study group. It is quite unlikely that any infants with stroke, presenting with seizures or altered mental status were missed, because neuroradiological investigations are routinely performed in such cases in Estonia.

Acute focal signs were present in 64 percent of IPH cases in the study, which is more than reported in previous studies (13 percent to 50 percent) (*Al-Jarallah et al., 2000; Meyer-Heim et al., 2003; Beslow et al., 2010*). Lo et al. (2008) have showed that neurological deficits are more common among children of at least six years of age than they are in younger children. This may partly explain the high percentage of focal neurological deficits among our cases of IPH, as children suffering from IPH in the present study were older than in studies by Meyer-Heim et al. (2003) and Beslow et al. (2010). In the present study,

71 percent of children with IPH were at least six years of age, compared to 59 percent in Mayer-Heim's study. Thirty-six percent of patients with IPH had seizures at the onset, which is within the previously reported range (27–41 percent) (*Al-Jarallah et al., 2000; Meyer-Heim et al., 2003; Beslow et al., 2010*).

6.3. Risk factors of paediatric stroke

6.3.1. Risk factors of perinatal stroke

This study revealed risk factors that had been previously identified (up to 2007) for perinatal stroke in 32 percent of children. When risk factors of perinatal stroke were reanalysed based on the data from recent case-control studies (*Benders et al., 2007; Chabrier et al., 2010; Simchen et al., 2009*), risk factors were found in 87 percent of children with perinatal stroke.

The question remains as to why some children with perinatal stroke are symptomatic in the newborn period and others are not. A comparison between the two study subgroups revealed that several perinatal factors that had previously been associated with perinatal vascular brain damage (*Lynch et al., 2002; Golomb et al., 2001; Lynch et al., 2005; Stelmach et al., 2005*), including placental pathology and perinatal asphyxia, were significantly more frequent among children with early diagnosis. Only prothrombotic factors were more common – although not significantly – among children with presumed perinatal stroke. This reflects the fact that inherited disorders, rather than adverse events around the delivery, play an important role in the pathogenesis of presumed perinatal stroke.

6.3.2. Risk factors of childhood stroke

Risk factors were identified in 85 percent of children with cerebrovascular disease in the study, 87.5 percent of AIS, 81 percent of haemorrhagic stroke and 100 percent of SVT cases. The proportion of detected risk factors is similar to previous studies, although not all patients were investigated thoroughly.

Ganesan et al. (2003) found that 46 percent of AIS patients had a previously known underlying predisposing condition for AIS. The data in the current study shows that underlying predisposing cause was found in 31 percent children with a cerebrovascular event and in 38 percent of children with AIS. It can be concluded from this data that preventive strategies can be planned in a considerable proportion of childhood stroke cases. This is especially important in cases of head trauma and acute head and neck infections, vasculitis and diseases that cause acquired thrombophilic states (such as nephrotic syndrome). In the case of previously healthy children without predisposing conditions,

15

feasible risk factor identification (cardiac disease, vasculopathy, coagulopathy) makes it possible to plan secondary prevention for recurrent cerebrovascular events.

The amount of small vessel disease is unknown in the present study because MRA is liable to miss the detection of small vessel abnormalities (*Husson et al., 2002; Benseler et al., 2006*) and because catheter angiography was not performed. At the same time, although the importance of small vessel disease in childhood stroke is unknown, isolated involvement of small arteries has been estimated to be a rare cause of arteriopathy in childhood stroke (*Husson et al., 2002*).

A recent study has reported that the risk of ischemic stroke is 5.4 for every 1000 children who undergo a cardiac operation (*Domi et al., 2008*). The present study did not find any cerebrovascular complications after cardiac surgery during the study period, although the discharge database of cardiac surgery clinic was searched and the child cardiologist of cardiac surgery clinic was personally contacted. This may be due to the small number of cardiac operations (approximately 40 per year) in Estonia. It is possible that some TIA cases were missed and that cardiac surgeons did not code subclinical stroke cases.

Sickle cell disease is a major risk factor for childhood stroke but it is very rare in Estonia. Unfortunately, not all children in this study were tested for arteriopathies and cardiac disorders and, as a result, the actual stroke aetiology profile may be different.

Trauma preceded AIS in seven of the children in this study. Three of these had major head trauma (inflicted brain injury and cerebral contusion) and four had mild head trauma. Ischemic lesions may develop in up to 50 percent of cases of inflicted traumatic injury (*Bonnier et al., 2003*) but the exact pathophysiologic mechanism is unknown. This may be the result of an indirect brain and neck injury causing arterial wall dissection. In addition, fat embolism, a complication of long-bone fractures, has been suggested (*Bonnier et al., 2003*). Because MRA from extra-and intracranial arteries were not performed on children with trauma, it is not possible to confirm or exclude the possible mechanism of arterial dissection in these children.

In the cohort of Lo et al. (2008), the proportion of brain tumours and congenital heart disease should be markedly increased among children with intracranial haemorrhage due to the widespread use of contemporary imaging techniques and improved care of chronic illnesses. None of the children with haemorrhagic stroke in the present study suffered from brain tumour or congenital heart disease. It is possible that some cases of subtle clinical manifestations, occurring together with more pronounced clinical manifestation, such as brain tumour or cardiac surgery, have not diagnosed by treating physicians. Regardless, none of those children had marked residual neurological deficit; if they had, they would have reached the paediatric neurologist's point of view.

6.4. Neurological outcome of perinatal stroke

Reports of neonates with stroke presenting with seizures but without significant birth asphyxia and encephalopathy have demonstrated favorable neurodevelopmental outcome (*Schulzke et al., 2005; Jan and Camfield, 1998; Fujimoto et al., 1992*). On the other hand, neurodevelopmental outcome was abnormal in all patients with neonatal stroke presenting symptoms of encephalopathy in a cohort study (*Ramaswamy et al., 2004*). The present study revealed that neonatal stroke is more likely to be followed by a severe stage of hemiparesis ($P<0.05$) than a presumed perinatal stroke. This is consistent with a previous finding that more severe symptoms in the newborn period correlate with significant disabilities at long-term follow-up (*Sreenan et al., 2000*).

The outcome study found that, apart from the persistent hemiparesis, one-third of cases developed epilepsy with the same occurrence among children with early and delayed diagnosis. An earlier study found that 35 percent of children with a hemiparetic form of cerebral palsy develop epilepsy (*Talvik et al., 1987*). This contradicts a previous study, in which infants with neonatal stroke were more likely to develop epilepsy (*Wu et al., 2004*). According to previous studies, postneonatal epilepsy occurs in 37–67 percent of children with neonatal stroke (*Lee et al., 2005; Golomb et al., 2007*) and in 23–42 percent of patients with presumed perinatal stroke (*Golomb et al., 2001; Lee et al., 2005; Kirton et al., 2008; Fitzgerald et al., 2007*). This variability in the prevalence of epilepsy may depend on the age of the children in the outcome studies. The median age of epilepsy in the present study was 26 months, which is later than the 16 months found by Golomb et al. (2007). As with previous studies, complex partial seizures were most common type of epilepsy developed after perinatal ischemic stroke (*Golomb et al., 2001*).

6.5. Time lag to diagnosis of childhood arterial ischemic stroke

This study supports the conclusions from previous studies that the diagnosis of childhood AIS is often delayed (*Gabis et al., 2002; McGlennan and Ganesan, 2008; Rafay et al., 2009a*). The total median delay to AIS diagnosis in the present study was 132 hours, which is much longer than the 20–22.7 hours of previous studies (*Gabis et al., 2002; Rafay et al., 2009a*). One reason for such a sizeable difference is that Rafay's study excluded children with a time delay of over 14 days, while the present study did not. In addition, the longest time delay in Gabis' study was 300 hours, compared to 1440 hours in the present study.

The time delay from symptom onset to initial medical assessment (including ambulance) was in 3.3 hours in the current study, which is similar to the 1.7–9 hours of previous studies (*Gabis et al., 2002; Rafay et al., 2009a*). In a study of

adult stroke patients, the patients reached the emergency department with an average delay of three hours from the onset of initial symptoms (*Agyeman et al., 2006*), although that study excluded patients who arrived later than 48 hours after a stroke. In Estonia, 36 percent of adult patients with brain infarct arrive at the hospital within three hours (*Vibo, 2007*). This data indicates that parents in Estonia are quite quick to seek help for their children with acute neurological problems.

The longest delay in this study was from the first neuroimaging to the final diagnosis of AIS (median 114 hours). The diagnosis of AIS was considered by the first physician in 50 percent of the cases in the present study and in 59 percent of cases, the first neuroimaging was normal or was indicative of another disease. This data is very similar to previous findings (*Rafay et al., 2009a; Braun et al., 2006*). Early CT is often negative (*Rafay et al., 2009a*), but this difficulty may be overcome with early use of MRI, diffusion images and MRA (*Roach et al., 2008*). The main reason why physicians did not hurry to order a confirming MRI was that there was no practice of using specific treatment for childhood AIS in earlier years. Guidelines for the management of childhood stroke have since been published (*Roach et al., 2008; Monagle et al., 2008*) that urge physicians to make an early diagnosis of AIS in children in order to start with early treatment.

Another reason for the time delay could be the subacute onset of symptoms (*Rafay et al., 2009a*). Several cases of childhood ischemic stroke, in contrast to adult stroke, present with subacute symptoms that cause the delayed diagnosis in the first place (*Braun et al., 2006; Meyer-Heim et al., 2003).*

6.6. Association between factor V Leiden and prothrombin 20210G>A mutation and paediatric ischemic stroke

This study revealed a significant association between FVL and PT 20210G>A mutations and SVT in children (OR: 12.9 and 11.9, respectively). Only one out of five previous studies showed such an association (*Heller et al., 2003*). As the results of most previous case-control studies conflicted with the present case-control study, it was felt that a meta-analysis on FVL/PT 20210G>A and paediatric SVT was required.

The meta-analysis on FVL and paediatric SVT suggested that FVL increases the risk of SVT in children and newborns 3.1-fold (95% CI: 1.8–5.5). The result is in accordance with a meta-analysis of 1430 children with venous thromboembolism of different locations, including cerebral venous thromboembolism, which showed a 3.5-fold increased risk of the first episode of venous thromboembolism among children with FVL (95% CI: 2.57–4.93) (*Young et al., 2008*). According to an adult population meta-analysis, the risk of

cerebral venous thrombosis is 3.4-fold higher among FVL carriers (95% CI: 2.27–5.05) (*Dentali et al., 2006*).

The meta-analysis on PT 20210G>A and paediatric SVT suggests that PT 20210G>A increases the risk of SVT in children and newborns 3.1-fold (95% CI: 1.4–6.8). This result is contradictory to previous case-control studies and the main reason for this may be the small study groups with large CIs. The result of the current meta-analysis is similar to the previous meta-analysis on venous thromboembolism of different locations, which reported 2.6-fold increased risk among children with PT 20210G>A mutation (95% CI: 1.6–4.41) (*Young et al., 2008*). The adult population meta-analysis reported a much higher OR (9.27) of developing cerebral venous thrombosis among patients with PT 20210G>A mutation (*Dentali et al., 2006*).

Meta-analyses performed separately on neonatal and childhood SVT failed to show a significant association between childhood SVT and FVL and between neonatal SVT and PT 20210G>A mutation. However, there was a strong trend towards the association in both cases (OR=2.3; 95% CI: 0.8–6.3 and OR=3.1, 95% CI: 0.8–12.4, respectively). The main reason for this could be the small number of the included studies that caused large CIs. It can be concluded that more case-control studies on genetic risk factors of childhood and neonatal SVT are needed in order to shed more light on this issue.

The study did not reveal an association between AIS and FVL or PT 20210G>A mutation. The data regarding this issue in the literature is controversial. The discrepancies may be caused by small study groups, which could lead to large CIs, a lack of controls, and technical variability. The most significant positive correlation between childhood AIS and FVL was found in the study with the largest number of AIS patients among case-control studies by German investigators (*Nowak-Göttl et al., 1999*). They found that children with FVL are at six times greater risk (95% CI: 2.98–8.03) of developing childhood AIS. At the same time, the prevalence of FVL among the control population was twice (six percent) as high as in the present study (three percent). This data indicates considerable variance between countries and may reflect ethnic diversities, even among European populations.

At least three meta-analyses have been published that assess the association between childhood AIS and FVL and/or PT 20210G>A mutation; unfortunately, these also provide contradictory results. Chan et al. (2000) performed a meta-analysis of five case-control studies of childhood AIS and reported a pooled OR of 4.3 (95% CI: 2.8–6.5) for FVL. Another meta-analysis of 453 children with AIS, by Juul et al. (2002), reported a pooled OR for FVL of 4.79 (95% CI: 3.26–7.03). Haywood et al. (2005) reported a meta-analysis of over 500 children, which suggested that FVL and PT 20210G>A are not associated with increased risk for childhood AIS. A meta-analyses of over 3000 adult cases by Casas et al. (2005) revealed a statistically significant association between AIS and both FVL (OR 1.33; 95% CI: 1.12–1.15) and PT 20210G>A mutation (OR 1.44; 95% CI: 1.11–1.86).

Although the results of the present study regarding perinatal and childhood AIS show no association between stroke and FVL or PT 20210G>A mutation, it is not recommended that testing of newborns and children with AIS for these mutations should stop. Firstly, the data in the literature are conflicting and no meta-analysis of exclusively perinatal AIS and FVL/PT 20210G>A mutation has been published. Secondly, there is an increasing understanding that the coexistence of several prothrombotic factors or trombophilia with other risk factors, as opposed to a single prothrombotic factor, is required in order to develop stroke in children (*Amlie-Lefond et al., 2008*). Thirdly, according to recent treatment guidelines (*Roach et al., 2008; Monagle et al., 2008*), initiation and/or prolongation of anticoagulant therapy depends on the occurrence of a prothrombotic factor in a newborn or a child with AIS.

One particular patient with SVT was double heterozygous to FVL and PT 20210G>A, and also homozygous for MTHFR 1298A>C polymorphism. Patients who are double heterozygous to FVL and PT 20210G>A have a 2.6 times greater risk of recurrent venous thrombosis than carriers of FVL alone (*De Stefano et al., 1999*). Nearly half (48.4 percent) of the children with first spontaneous venous thromboembolism and multiple prothrombotic defects experience recurrent venous thromboembolism (*Nowak-Göttl et al., 2001b*). This data stresses the need for anticoagulant therapy for children who have suffered from venous thromboembolism and have multiple prothrombotic risk factors in situations that predispose them to recurrent venous thromboembolism (for example, immobilisation, surgery, central venous lines, smoking). When consulting with teenage female patients, more attention should be paid to the usage of oral contraceptives, increased risk of thrombosis during pregnancy and the enhanced risk of developing other pregnancy complications.

This is the first report from Eastern Europe for both FVL and PT 20210G>A mutations. The reported FVL allele frequency of 1.5 percent in the general Estonian population is more infrequent than the 2.7 percent reported for all of Europe (*Rees, 1996*). FVL is very rare in individuals of Asian and African descent. Estonia's closest neighbours, Finland and Russia (Saint Petersburg), have the same FVL allele frequency as found in the present study (1.6 percent) (*Rees, 1996; Tadtaeva et al., 2007*).

The present study reports a PT 20210G>A allele frequency of 1.6 percent in the general Estonian population. The total PT 20210G>A allele frequency is 1.0 percent in Europe (*Rosendaal et al., 1998*). There is a North to South gradient of PT 20210G>A distribution within Europe, with the allele frequency being nearly twice as high in Southern Europe (1.5 percent) as it is in Northern Europe (0.8 percent) (*Rosendaal et al., 1998*). Estonia's location, in North-East Europe, means that the allele frequency of 1.6 percent is higher than expected. In Russia (Saint Petersburg), the reported PT 20210G>A allele frequency is even higher, at 2.1 percent (*Tadtaeva et al., 2007*).

7. CONCLUSIONS

1. The incidence rate of perinatal stroke in Estonia is 63/100,000, or 1/1578 live births, which is more than estimated in previous studies in other countries. The incidence rate of childhood stroke in Estonia is 2.73/100,000, which is similar to earlier reported data.
2. One-third of patients with perinatal stroke have symptoms after birth (seizures in 92 percent of cases). The remaining two-thirds reach medical attention at a mean age of eight months, mostly (85 percent) because of hemiparesis. Focal neurological symptoms occur in all patients with childhood AIS, in 64 percent of patients with childhood haemorrhagic stroke and in half of the children with SVT.
3. Risk factors for perinatal stroke and childhood stroke are found in the majority of children and are very diverse. According to this study, the most frequently identified risk factors of perinatal stroke are primiparity, emergent caesarean section, an Apgar score <7 at the first minute, preeclampsia and prothrombotic factors. Arteriopathy, cardiac disorder and prothrombotic factors are the main risk factors of childhood AIS.
4. Hemiparesis occurs in all children with perinatal stroke at outcome and is moderate or severe in 76 percent of children. Epilepsy develops in one-third of children, both with neonatal and presumed perinatal stroke.
5. The diagnosis of childhood AIS is often delayed. The main delay occurs from initial neuroimaging to the final diagnosis, mainly because the initial neuroimaging is frequently nonindicative for AIS.
6. FVL and PT 20210G>A increase the risk of paediatric SVT 3.1 times. Investigation of these mutations is indicated in neonates and children with SVT.

8. REFERENCES

Agyeman O, Nedeltchev K, Arnold M, Fischer U, Remonda L, Isenegger J, Schroth G, Mattle HP. Time to admission in acute ischemic stroke and transient ischemic attack. Stroke 2006; 37: 963–966.

Akar N, Akar E, Özel D, Deda G, Sipahi T. Common mutations at the homocysteine metabolism pathway and pediatric stroke. Thromb Res 2001; 102: 115–20.

Akar N, Akar E, Deda G, Sipahi T, Orsal A. Factor V1691 G–A, prothrombin 20210 G-A, and methylenetetrahydrofolate reductase 677 C-T variants in Turkish children with cerebral infarct. J Child Neurol 1999; 14: 749–751.

Albers GW, Thijs VN, Wechsler L, Kemp S, Schlaug G, Skalabrin E, Bammer R, Kakuda W, Lansberg MG, Shuaib A, Coplin W, Hamilton S, Moseley M, Marks MP, for the DEFUSE Investigators. Magnetic Resonance Imaging Profiles Predict Clinical Response to Early Reperfusion: The Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE) Study. Ann Neurol 2006; 60: 508–517.

Al-Jarallah A, Al-Rifai MT, Riela AR, Roach ES. Nontraumatic brain hemorrhage in children: Etiology and presentation. J Child Neurol 2000; 15: 284–289.

Amlie-Lefond C, Sébire G, Fullerton HJ. Recent developments in childhood arterial ischaemic stroke. Lancet Neurol 2008; 7: 425–435.

Amlie-Lefond C, Bernard TJ, Sébire G, Friedman NR, Heyer GL, Lerner NB, deVeber G, Fullerton HJ, for the International Pediatric Stroke Study Group. Predictors of cerebral arteriopathy in children with arterial ischemic stroke. Circulation 2009; 119: 1417–1423.

Armstrong-Wells J, Johnston SC, Wu YW, Sidney S, Fullerton HJ. Prevalence and predictors of perinatal hemorrhagic stroke: results from the Kaiser pediatric stroke study. Pediatrics 2009; 123: 823–828.

Aronis S, Bouza H, Pergantou H, Kapsimalis Z, Platokouki H, Xanthou M. Prothrombotic factors in neonates with cerebral thrombosis and intraventricular hemorrhage. Acta Paediatr Suppl 2002; 438: 87–91.

Askalan R, Laughlin S, Mayank S, Chan A, MacGregor D, Andrew M, Curtis R, Meaney B, deVeber G. Chickenbox and Stroke on childhood. Stroke 2001; 32: 1257.

del Balzo F, Spalice A, Ruggieri M, Greco F, Properzi E, Iannetti P. Sroke in children: inherited and aquired factors and age-related variations in the presentation of 48 pediatric patients. Acta Paediatr 2009; 98: 1130–1136.

Barnes C, Newall F, Furmedge J, Mackay M, Monagle P. Arterial ischaemic stroke in children. J Paediatr Child Health 2004; 40: 384–387.

Barnes C, Newall F, Furmedge J, Mackay M, Monagle P. Cerebral sinus venous thrombosis in children. J Paediatr Child Health 2004; 40: 53–55.

Barreirinho S, Ferro A, Santos M, Costa E, Pinto-Basto J, Sousa A, Sequeiros J, Maciel P, Barbot C, Barbot J. Inherited and aquired risk factors and their combined effects in pediatric stroke. Pediatr Neurol 2003; 28: 134–138.

Bassan H, Feldman HA, Limperopoulos C, Benson CB, Ringer SA, Veracruz E, Soul JS, Volpe JJ, du Plessis AJ. Periventricular hemorrhagic infarction: Risk factors and neonatal outcome. Pediatr Neurol 2006; 35: 85–92.

Benders MJ, Groenendaal F, Uiterwaal CS, Nikkels PG, Bruinse HW, Nievelstein RA, de Vries LS. Maternal and infant characteristics associated with perinatal arterial stroke in the preterm infant. Stroke 2007; 38: 1759–1765.

Benders MJ, Groenendaal F, Uiterwaal CS, de Vries LS. Perinatal arterial stroke in the preterm infant. Semin Perinatol 2008; 32: 344–349.
Benedict S, Bonkowsky JL, Thompson JA, Van Orman CB, Boyer RS, Bale JF, Filloux FM. Cerebral sinovenous thrombosis in children: another reason to treat iron deficiency anemia. J Child Neurol 2004; 19: 526–531.
Benseler SM, Silverman E, Aviv RI, Schneider R, Armstrong D, Tyrrell PN, DeVeber G. Primary CNS vasculitis in children. Arthritis Rheum 2006; 54: 1291–1297.
Bergman I, Bauer RE, Barmada MA, Latchaw RE, Taylor HG, David R, Painter MJ. Intracerebral hemorrhage in the full-term neonatal infant. Pediatrics 1985; 75: 488–496.
Bernard and Goldenberg. Pediatric arterial ischemic stroke. Pediatr Clin N Am 2008; 55: 323–338.
Beslow LA, Licht DJ, Smith SE, Storm PB, Heuer GG, Zimmerman RA, Feiler AM, Kasner SE, Ichord RN, Jordan LC. Predictors of outcome in childhood intracerebral hemorrhage: a prospective consecutive cohort study. Stroke 2010; 41: 313–318.
van Beynum IM, Smeitink JA, den Heijer M, te Poele Pothoff MT, Blom HJ. Hyperhomocysteinemia: a risk factor for ischemic stroke in children. Circulation 1999; 99: 2070–2072.
Boardman JP, Ganesan V, Rutherford MA, Saunders DE, Mercuri E, Cowan F. Magnetic resonance image correlates of hemiparesis after neonatal and childhood middle cerebral artery stroke. Pediatrics 2005; 115: 321–326.
Bonduel M, Sciuccati G, Hepner M, Pieroni G, Torres AF, Mardaraz C, Frontroth JP. FVL and prothrombin gene G20210A mutation in children with cerebral thromboembolism. Am J Hematol 2003; 73: 81–86.
Bonduel M, Sciuccati G, Hepner M, Pieroni G, Torres AF, Frontroth JP, Tenembaum S. AIS and cerebral venous thrombosis in children: A 12-year Argentinean registry. Acta Haematol 2006; 115: 180–185.
Bonnier C, Nassogne M-C, Saint-Martin C, Mesples B, Kadhim H, Sébire G. Neuroimaging of intraparenchymal lesions predicts outcome in shaken baby syndrome. Pediatrics 2003; 112: 808–814.
Braun KPJ, Kappelle LJ, Kirkham F, DeVeber G. Diagnostic pitfalls in paediatric ischaemic stroke. Dev Med Child Neurol 2006; 48: 985–990.
Braun KPJ, Rafay MF, Uiterwaal CSPM, Pontigon A-N, DeVeber G. Mode of onset predicts etiological diagnosis of AIS in children. Stroke 2007; 38: 298–302.
Braun KP, Bulder MM, Chabrier S, Kirkham FJ, Uiterwaal CS, Tardieu M, Sébire G. The course and outcome of unilateral intracranial arteriopathy in 79 children with ischaemic stroke. Brain 2009; 132: 544–557.
Broderick J, Talbot GT, Prenger E, Leach A, Brott T. Stroke in children within a major metropolitan area: the surprising importance of intracerebral hemorrhage. J Child Neurol 1993; 8: 250–255.
Broderick J, Connolly S, Feldmann E, Hanley D, Kase C, Krieger D, Mayberg M, Morgenstern L, Ogilvy CS, Vespa P, Zuccarello M; American Heart Association; American Stroke Association Stroke Council; High Blood Pressure Research Council; Quality of Care and Outcomes in Research Interdisciplinary Working Group. Guidelines for the management of spontaneous intracerebral hemorrhage in adults: 2007 update: a guideline from the American Heart Association/American Stroke Association Stroke Council, High Blood Pressure Research Council, and the Quality of Care and Outcomes in Research Interdisciplinary Working Group. Stroke 2007; 38: 2001–2023.

Burger IM, Murphy KJ, Jordan LC, Tamargo RJ, Gailloud P. Safety of cerebral digital subtraction angiography (DSA) in children: Complication rate analysis in 241 consecutive diagnostic angiograms. Stroke 2006; 37: 2535–2539.

Carvalho KS, Bodensteiner JB, Connolly PJ, Garg BP. Cerebral venous thrombosis in children. J Child Neurol 2001; 16: 574–580.

Casas JP, Hingorani AD, Bautista LE, Sharma P. Meta-analysis of genetic studies in ischemic stroke: thirty-two genes involving approximaterly 18 000 cases and 58 000 controls. Arch Neurol 2004; 61: 1652–1662.

Chabrier S, Saliba E, Nguyen The Tich S, Charollais A, Varlet MN, Tardy B, Presles E, Renaud C, Allard D, Husson B, Landrieu P**.** Obstetrical and neonatal characteristics vary with birthweight in a cohort of 100 term newborns with symptomatic arterial ischemic stroke. Eur J Paediatr Neurol 2010; 14: 206–13.

Chadehumbe MA, Khatri P, Khoury JC, Alwell K, Szaflarski JP, Broderick JP, Kissela BM, Kleindorfer DO. Seizures are Common in the Acute Setting of Childhood Stroke: A Population-Based Study. J Child Neurol 2009; 24: 9–12.

Chan A, Crowther M, deVeber G. Factor V Leiden and arterial ischemic stroke: a metanalysis. Blood 2000; 95(Suppl 1): 645(abstr.).

Chung B, Wong V. Childhood stroke among Hong Kong Chinese subjects. Pediatrics 2004; 114: e206–12.

Cowan F, Rutherford M, Groenendaal F, Ekel P, Mercuri E, Bydder GM, Meiners LC, Dubowitz LMS, de Vries LS. Origin and timing of brain lesions in term infants with neonatal encefalopathy. The Lancet 2003; 361: 736–742.

Cowan F, Mercuri R, Groenendaal F, Bassi L, Ricci D, Rutherford M, de Vries L. Does cranial ultrasound imaging identify arterial cerebral infarction in term neonates? Arch Dis Child Fetal Neonatal Ed 2005; 90: F252–F256.

D'Arceuil H, Rhine W, de Crespigny A, Yenari M, Tait JF, Strauss WH, Engelhorn T, Kastrup A, Moseley M, Blankenberg FG. 99mTc annexin V imaging of neonatal hypoxic brain injury. Stroke 2000; 31: 2692–2700.

Dentali F, Crowther M, Ageno W. Thrombophilic abnormalities, oral contraceptives, and risk of cerebral vein thrombosis: a meta-analysis. Blood 2006; 107: 2766–2773.

Dlamini N, Kirkham FJ. Stroke and cerebrovascular disorders. Curr Opin Pediatr 2009; 21: 751–761.

Domi T, Edgell DS, McCrindle BW, Williams WG, Chan AK, MacGregor DL, Kirton A, deVeber GA. Frequency, predictors, and neurologic outcomes of vaso-occlusive strokes associated with cardiac surgery in children. Pediatrics 2008; 122: 1292–8.

Duering C, Kosch A, Langer C, Thedieck S, Nowak-Göttl U. Total tissue factor pathway inhibitor is an independent risk factor for symptomatic paediatric venous thromboembolism and stroke. Thromb and Haemost 2004; 92: 707–712

Duran R, Biner B, Demir M, et al. Factor V Leiden mutation and other thrombophilia markers in childhood ischemic stroke. Clin Appl Thrombosis/Hemostasis. 2005; 11: 83–88

Earley CJ, Kittner SJ, Feeser BR, Gardner J, Epstein A, Wozniak MA, Wityk R, Stern BJ, Price TR, Macko RF, Johnson C, Sloan MA, Buchholz D. Stroke in children and sickle-cell disease. Baltimore-Washington Cooperative Young Stroke Study. Neurology 1998; 51: 169–76.

Eeg-Olofsson O, Ringheim Y. Stroke in children. Clinical characteristics and prognosis. Acta Paediatr 1983; 72: 391–395.

Elalfy M, Elbarbary N, Khaddah N, Abdelwahab M, El Rashidy F, Hassab H, Al-Tonbary Y. Intracranial hemorrhage in acute and chronic childhood immune

thrombocytopenic purpura over a ten-year period: an Egyptian multicenter study. Acta Haematol 2010; 123: 59–63.
Emam AT, Ali AM, Babikr MA. Childhood stroke in Eastern Province, KSA: pattern, risk factors, diagnosis and outcome. Acta Paediatr 2009; 98: 1613–1619.
Estan J, Hope P. Unilateral neonatal cerebral infarction in full term infants. Arch Dis Child Fetal Neonatal Ed 1997; 76: F88–F93.
Estonian Statistics. Available at http://pub.stat.ee/
Fitzgerald KC, Williams LS, Garg BP, Carvalho KS, Golomb MR. Cerebral Sinovenous Thrombosis in the Neonate. Arch Neurol 2006; 63: 405–409.
Fitzgerald KC, Williams LS, Garg BP, Golomb MR. Epilepsy in children with delayed presentation of perinatal stroke. J Child Neurol 2007; 22: 1274–1280.
Fujimoto S, Yokochi K, Togari H, Nishimura Y, Inukai K, Futamura M, Sobajima H, Suzuki S, Wada Y. Neonatal cerebral infarction: Symptoms, CT findings and prognosis. Brain Dev 1992; 14: 48–52.
Fullerton HJ, Johnston SC, Smith WS. Arterial dissection and stroke in children. Neurology 2001; 57: 1155–1160.
Fullerton HJ, Chetkovich DM, Wu YW, Smith WS, Johnston SC. Deaths from stroke in US children, 1979 to 1998. Neurology 2002; 59: 34–9.
Fullerton HJ, Wu YW, Zhao S, Johnston SC. Risk of stroke in children: Ethnic and gender disparities. Neurology 2003; 61: 189–94.
Fullerton HJ, Adams RJ, Zhao S, Johnston SC. Declining stroke rates in Californian children with sickle cell disease. Blood 2004; 104: 336–339.
Fullerton HJ, Wu YW, Sidney S, Johnston C. Risk of recurrent childhood arterial ischemic stroke in a population-based cohort: the importance of cerebrovascular imaging. Pediatrics 2007; 119: 495–501.
Fullerton HJ, Wu YW, Sidney S, Johnston SC. Recurrent hemorrhagic stroke in children: a population-based cohort study. Stroke 2007; 38: 2658–2662.
Fung E, Ganesan V, Cox TS, Chong WK, Saunders DE. Complication rates of diagnostic cerebral arteriography in children. Pediatr Radiol 2005; 35: 1174–1177.
Gabis LV, Yangala R, Lenn NJ. Time lag to the diagnosis of stroke in children. Pediatrics 2002; 110: 924–928.
Ganesan V, McShane MA, Liesner R, Cookson J, Hann I, Kirkham FJ. Inherited prothromotic states and ishaemic stroke in childhood. J Neurol Neurosurg Psychiatry 1998; 65: 508–11.
Ganesan V, Hogan A, Shack N, Gordon A, Isaacs E, Kirkham FJ. Outcome after ischaemic stroke in childhood. Dev Med Child Neurol 2000; 42: 455–461.
Ganesan V, Prengler M, McShane MA, Wade AM, Kirkham FJ. Investigation of risk factors in children with arterial ischemic stroke. Ann Neurol 2003; 53: 167–73.
Gardner MA, Hills NK, Sidney S, Johnston SC, Fullerton HJ. The 5-year direct medical cost of neonatal and childhood stroke in a population-based cohort. Neurology 2010; 74: 372–378.
Gentilomo C, Franzoi M, Laverda AM, Suppiej A, Battistella PA. Cerebral sinovenous thrombosis in children: thrombophilia and clinical outcome. Thromb Res 2008; 121: 589–591.
Giroud M, Lemesle M, Gouyon JB, Nivelon JL, Milan C, Dumas R. Cerebrovascular disease in children under 16 years of age in the city of Dijon, France: a study of incidence and clinical features from 1985 to 1993. J Clin Epidemiol 1995; 48: 1343–1348.

Giroud M, Lemesle M, Madinier G, Manceau E, Osseby GV, Dumas R. Stroke in children under 16 years of age. Clinical and etiological difference with adults. Acta Neurol Scand 1997; 96: 401–406.
Golomb MR, MacGregor DL, Domi T, Armstrong DC, McCrindle BW, Mayank S, deVeber GA. Presumed pre- or perinatal arterial ischemic stroke: risk factors and outcomes. Ann Neurol 2001; 50:163–168.
Golomb MR, Dick PT, MacGregor DL, Armstrong DC, deVeber GA. Cranial ultrasonography has a low sensitivity for detecting AIS in term neonates. Journal of Child Neurol 2003; 18: 98–103.
Golomb MR, deVeber GA, MacGregor DL, Domi T, Whyte H, Stephens D, Dick PT. Independent walking after neonatal AIS and sinovenous thrombosis. J Child Neurol 2003; 18: 530–536.
Golomb MR, Dick PT, MacGregor DL, Curtis R, Sofronas M, deVeber GA. Neonatal arterial ischemic stroke and cerebral sinovenous thrombosis are more commonly diagnosed in boys. J Child Neurol 2004; 19: 493–497.
Golomb MR, Garg BP, Carvalho KS, Johnson CS, Williams LS. Perinatal stroke and the risk of developing childhood epilepsy. J Pediatr 2007; 151: 409–413.
Golomb MR, Garg BP, Edwards-Brown M, Williams LS. Very early AIS in premature infants. Pediatr Neurol 2008; 38: 329–334.
Golomb MR, Garg BP, Saha C, Azzouz F, Williams LS. Cerebral palsy after perinatal arterial ischemic stroke. J Child Neurol 2008; 23: 279–286.
Golomb MR, Fullerton HJ, Nowak-Gottl U, DeVeber G; for the International Pediatric Stroke Study Group. Male predominance in Childhood Ischemic Stroke. Findings from the International Pediatric Stroke Study. Stroke 2009; 40: 52–57.
Golomb MR. Outcomes of perinatal AIS and cerebral sinovenous thrombosis. Semin Fetal Neonatal Med 2009; 14: 318–322.
González-Bonet LG, Ollero-Ortiz A, Giménez-Pando J, Márquez-Rivas J. Neonatal intracranial haemorrhage secondary to spontaneous rupture of an aneurysm. Illustrative case and literature review. Rev Neurol 2010; 50: 403–408.
Govaert P, Matthys E, Zecic A, Roelens F, Oostra A, Vanzieleghem B. Perinatal cortical infarction within middle cerebral artery trunks. Arch Dis Child Fetal Neonatal Ed 2000; 82: F59–F63.
Govaert P, Ramenghi L, Taal R, de Vries L, deVeber G. Diagnosis of perinatal stroke I: definitions, differential diagnosis and registration. Acta Paediatr 2009; 98: 1556–1567.
Govaert P, Ramenghi L, Taal R, Dudnik J, Lequin M. Diagnosis of perinatal stroke II: mechanisms and clinical phenotypes. Acta Paediatr 2009; 98: 1720–1726.
Günther G, Junker R, Sträter R, Schobess R, Kurnik K, Nowak-Göttl U. Symptomatic ischemic stroke in full-term neonates: role of aquired and genetic prothrombotic risk factors. Stroke 2000; 31: 2437–2441.
Hagstrom JN, Walter J, Bluebond-Langner R, Amatniek JC, Manno CS, High KA. Prevalence of the factor V Leiden mutation in children and neonates with thrombembolic disease. J Pediatr 1998; 133: 777–781.
Hatano S. Experience from a multicentre stroke register: a preliminary report. Bull World Health Organ 1976; 54: 541–553.
Haywood S, Liesner R, Pindora S, Ganesan V. Thrombophilia and first arterial ischaemic stroke: a systemic review. Arch Dis Child 2005; 90: 402–405.

Heller C, Heinecke A, Junker R, Knöfler R, Kosch A, Kurnik K, Schobess R, von Eckardstein A, Sträter R, Zieger B, Nowak-Göttl U. Cerebral venous thrombosis in children: a multifactorial origin. Circulation 2003; 108: 1362–1367.

Herak DC, Antolic MR, Krleza JL, Pavic M, Dodig S, Duranovic V, Brkic AB, Zadro R. Inherited prothrombotic risk factors in children with stroke, transient ischemic attack, or migraine. Pediatrics 2009; 123: e653–60.

Hogeveen M, Blom HJ, van Amerongen M, Boogmans B, van Beynum IM, van de Bor M. Hyperhomocysteinemia as risk factor for ischemic and hemorrhagic stroke in newborn infants. J Pediatr 2002; 141: 429–431.

Husson B, Rodesch G, Lasjaunias P, Tardieu M, Sébire G. Magnetic resonance angiography in childhood arterial brain infarcts: a comparative study with contrast angiography. Stroke 2002; 33: 1280–1285.

International Paediatric Stroke Study. Available at https://app3.ccb.sickkids.ca/cstrokestudy/. Accessed October 30, 2008. Version of June 15, 2007.

Jan MM, Camfield PR. Outcome of neonatal stroke in full-term infants without significant birth asphyxia. Eur J Pediatr 1998; 157: 846–848.

Jahwar BS, Ranger A, Steven D, Del Maestro RF. Risk factors for intracranial hemorrhage among full-term infants: A case-control study. Neurosurgery 2003; 52: 581–590.

Jordan LC, Hillis AE. Hemorrhagic Stroke in Children. Pediatric Neurology 2007; 36: 73–80.

Jordan LC, Johnston SC, Wu YW, Sidney S, Fullerton JF. The importance of cerebral aneurysms in childhood hemorrhagic stroke. A population-based study. Stroke 2009; 40: 400–405.

Jordan LC, Rafay MF, Smith SE, Askalan R, Zamel KM, Deveber G, Ashwal S; for the International Pediatric Stroke Study Group. Antithrombotic Treatment in Neonatal Cerebral Sinovenous Thrombosis: Results of the International Pediatric Stroke Study. J Pediatr 2010; 156: 704–710.

Juul K, Tybjaerg-Hansen A, Steffensen R, Kofoed S, Jensen G, Nordestgaard BG. Factor V Leiden: The Copenhagen city heart study and 2 meta-analyses. Blood 2002; 100: 3–10.

Kalafatis M, Rand MD, Mann KG. The Mechanism of inactivation of human factor V and human factor VA by activated protein C. J Biol Chem 1994; 269: 31869–31880.

Kenet G, Sadetzki S, Murad H, Martinowitz U, Rosenberg N, Gitel S, Rechavi G, Inbal A. Factor V Leiden and antiphospholipid antibodies are significant risk factors for ischemic stroke in children. Stroke 2000; 31: 1283–1288.

Kenet G, Waldman D, Lubetsky A, Kornbrut N, Khalil A, Koren A, et al. Paediatric cerebral sinus vein thrombosis A multi-center, case-controlled study. Thromb Haemost 2004; 92: 713–718.

Kenet G, Kirkham F, Niederstadt T, Heinecke A, Saunders D, Stoll M, Brenner B, Bidlingmaier C, Heller C, Knöfler R, Schobess R, Zieger B, Sébire G, Nowak-Göttl U. Risk factors for recurrent venous thrombembolism in the European collaborative paediatric database on cerebral venous thrombosis: a multicentre cohort study. Lancet Neurol 2007; 6: 595–603.

Kieslich M, Fiedler A, Heller C, Kreuz W, Jacobi G. Minor head injury as cause and co-factor in the aetiology of stroke in childhood: a report of eight cases. J Neurol Neurosurg Psychiatry 2002; 73: 13–16.

Kirkham FJ, Prengler M, Hewes DK, Ganesan V. Risk factors for arterial ischemic stroke in children. J Child Neurol 2000; 15: 299–307.
Kirkham F, Sébire G, Steinlin M, Sträter R. Arterial ischaemic stroke in children: Review of the literature and strategies for future stroke studies. Thromb and Haemost 2004; 92: 697–706.
Kirton A, deVeber G. Advances in perinatal ischemic stroke. Pediatr Neurol 2009; 40: 205–214.
Kirton A, deVeber G, Pontigon A-M, Macgregor D, Shroff M. Presumed perinatal ischemic stroke: vascular classification predicts outcome. Ann Neurol 2008; 63: 436–443.
Kleindorfer D, Khoury J, Kissela B, Alwell K, Woo D, Miller R, Schneider A, Moomaw C, Broderick JP. Temporal trends in the incidence and case fatality of stroke in children and adolescents. J Child Neurol 2006; 21: 415–418.
Komitopoulou A, Platokouki H, Kapsimali Z, Pergantou H, Adamtziki E, Aronis S. Mutations and polymorphisms in genes affecting hemostasis proteins and homocysteine metabolism in children with arterial ischemic stroke. Cerebrovasc Dis 2006; 22: 13–20.
Kurnik K, Kosch A, Sträter R, Scobess R, Heller C, Nowak-Göttl U. Recurrent thromboembolism in infants and children suffering from symptomatic neonatal arterial stroke. Stroke 2003; 34: 2887–2893.
Lanthier S, Carmant L, David M, Larbrisseau A, deVeber G. Stroke in children: the coexistence of multiple risk factors predicts poor outcome. Neurology 2000; 54: 371–378.
Lanthier S, Armstrong D, Domi T, deVeber G. Post-varicella arteriopathy of childhood: Natural history of vascular stenosis. Neurology 2005; 64: 660–663.
Laurichesse DH, Winer N, Gallot D, Lopes K, Perrotin F, Fluncker S, Geissler F, Beaufrere AM, Vendittelli F, Couture C, Lemery D. Prenatal diagnosis of thrombosis of the dural sinuses: report of six cases, review of the literature and suggested management. Ultrasound Obstet Gynecol 2008; 32: 188–198.
Lee J, Croen LA, Backstrand KH, Yoshida CK, Henning LH, Lindan C, Ferriero DM, Fullerton HJ, Barkovich AJ, Wu YW. Maternal and infant characteristics associated with perinatal arterial stroke in the infant. JAMA 2005; 293: 723–729.
Lee J, Croen LA, Lindan C, Nash KB, Yoshida CK, Ferriero DM, Barkovich AJ, Wu YW. Predictors of outcome in perinatal arterial stroke: A population-based study. Ann Neurol 2005; 58: 303–308.
Lee YY, Lin KL, Wang HS, Chou ML, Hung PC, Hsieh MY, Lin JJ, Wong AM. Craniocervical arterial dissection: a cause of childhood arterial ischemic stroke in Taiwan. J Formos Med Assoc. 2010; 109: 156–162.
Lindsberg PJ, Grau AJ. Inflammation and infections as risk factors for ischemic stroke. Stroke 2003; 34: 2518–2532.
Liu AC, Segaren N, Cox TS, Hayward RD, Chong WK, Ganesan V, Saunders DE. Is there a role for magnetic resonance imaging in the evaluation of non-traumatic intraparenchymal haemorrhage in children? Pediatr Radiol 2006; 36: 940–946.
Lo WD, Lee JE, Rusin J, Perkins E, Roach ES. Intracranial hemorrhage in children. Arch Neurol 2008; 65: 1629–1633.
Lucotte G, Champenois T. Duplex PCR-RFLP for simultaneous detection of factor V Leiden and prothrombin G20210A. Mol Cell Probes 2003; 17: 267–269.
Lutterman J, M Scott, R Nass and T Geva, Moyamoya syndrome associated with congenital heart disease. Pediatrics 1998; 101: 57–60.

Lynch JK, Hirtz DG, DeVeber G, Nelson KB. Report of the national institute of neurological disorders and stroke workshop on perinatal and childhood stroke. Pediatrics 2002; 109: 116–123.

Lynch JK, Han CJ, Nee LE, Nelson KB. Prothrombotic factors in children with stroke or porencephaly. Pediatrics 2005; 116: 447–453.

Lynch JK. Epidemiology and classification of perinatal stroke. Semin Fetal Neonatal Medicine 2009; 14: 245–249.

Mallick AA, Ganesan V. AIS in children – recent advances. Indian J Pediatr 2008; 75: 1149–1157.

Mallick AA, Sharples PM, Calvert SE, Jones RWA, Leary M, Lux AL, O'Callaghan FJ, Osborne JP, Patel JS, Prendiville AT, Renowden S, Jardine PE. Cerebral venous sinus thrombosis: a case series including thrombolysis. Arch Dis Child 2009; 94: 790–794.

Mallick AA, O'Callaghan FJK. The epidemiology of childhood stroke. Eur J Paediatr Neurol 2010; 14: 197–205.

Mallick AA, Ganesan V, O'Callaghan FJK. Mortality from childhood stroke in England and Wales, 1921–2000. Arch Dis Child 2010; 95: 12–19.

McGlennan C, Ganesan V. Dealys in investigation and management of acute arterial ischemic stroke in children. Devel Med Child Neurol 2008; 50: 537–540.

Meyer-Heim AD, Boltshauser E. Spontaneous intracranial haemorrhage in children: aetiology, presentation and outcome. Brain Dev 2003; 25: 416–421

Mercuri E, Rutherford M, Cowan F, Pennock J, Counsell S, Papadimitriou M, Azzopardi D, Bydder G, Dubowitz L. Early prognostic indicators of outcome in infants with neonatal cerebral infarction: A clinical, electroencephalogram, and magnetic resonance imaging study. Pediatrics 1999; 103: 39–46.

Mercuri E, Cowan F, Gupte G, Manning R, Laffan M, Rutherford M, Edwards AD, Dubowitz L, Roberts I. Prothrombotic disorders and abnormal neurodevelopmental outcome in infants with neonatal cerebral infarction. Pediatrics 2001; 107: 1400–1404.

Mercuri E, Barnett A, Rutherford M, Guzzetta A, Haataja L, Cioni G, Cowan F, Dubowitz L. Neonatal cerebral infarction and neuromotor outcome at school age. Pediatrics 2004; 113: 95–100.

Miller SP, Wu YW, Lee J, Lammer EJ, Iovannisci DM, Glidden DV, Bonifacio SL, Collins A, Shaw GM, Barkovich AJ, Ferriero DM. Candidate gene polymorphisms do not differ between newborns with stroke and normal controls. Stroke 2006; 37: 2678–2683.

Monagle P, Chalmers E, Chan A. Antithrombin therapy in neonates and children: American College of Chest Physicians evidence-based clinical practice guidelines (8th edition). Chest 2008; 133: 887–968.

Morita DC, Donaldson A, Butterfield RJ, Benedict SL, Bale JF. Methylenetetra-hydropholate reductase gene polymorphism and childhood stroke. Pediatr Neurol 2009; 41: 247–249.

Normann S, de Veber G, Fobker M, Langer C, Kenet G, Bernard TJ, Fiedler B, Sträter R, Goldenberg NA, Nowak-Göttl U. Role of endogenous testosterone concentration in pediatric stroke. Ann Neurol 2009; 66: 754–758.

Nowak-Göttl U, Junker R, Hartmeier M, Koch HG, Münchow N, assmann G, von Eckardstein A. Increased lipoprotein(a) in an important riks factor for venous thromboembolism in childhood. Circulation 1999; 100: 743–748.

Nowak-Göttl U, Sträter R, Kosch A, von Eckardstein A, Schobess R, Luigs P, Nabel P, Vielhaber H, Kurnik K, Junker R. The plasminogen activator inhibitor (PAI)-1 promoter 4G/4G genotype is not associated with ischemic stroke in a population of German children. Childhood Stroke Study Group. Eur J Haematol 2001; 66: 57–62.

Nowak-Göttl U, Junker R, Kreuz W, von Eckardstein A, Kosch A, Nohe N, et al, for the Childhood Thrombophilia Study Group. Risk of recurrent venous thrombosis in children with combined prothrombotic risk factors. Blood 2001; 97: 858–862.

Nwosu ME, Williams LS, Edwards-Brown M, Eckert GJ, Golomb MR. Neonatal sinovenous thrombosis: presentation and association with imaging. Pediatr Neurol 2008; 39: 155–161.

Ohene-Frempong K, SJ Weiner and LA Sleeper, Miller ST, Embury S, Moohr JW, Wethers DL, Pegelow CH, Gill FM, an the Cooperative study of Sickle cell disease. Cerebrovascular accidents in sickle cell disease: rates and risk factors. Blood 1998; 91: 288–294.

Ozyrek E, Balta G, Degerliyurt A, Parlak H, Aysun S, Gürgey A. Significance of factor V, prothrombin, MTHFR, and PAI-1 genotypes in childhood cerebral thrombosis. Clin Appl Thromb/Hemost 2007; 13: 154–160.

Pappachan J, Kirkham FJ. Cerebrovascular disease and stroke. Arch Dis Child 2008; 93: 890–898.

Perlman JM, Rollins NK, Evans D. Neonatal stroke: clinical characteristics and cerebral blood flow velocity measurements. Pediatr Neurol 1994; 4: 281–284.

Pilarska E, Lemka M, Bakowska A. Prothrombotic risk factors in ischemic stroke and migraine in cildren. Acta Neurol Scand 2006; 114: 13–16.

Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3' untranslated region of the prothrombin gene is associated to an elevated plasma prothrombin level and increase in venous thrombosis. Blood 1996; 88: 3698–3703.

Prengler M, Sturt N, Krywawych S, Surtees R, Liesner R, Kirkham F. Homozygous thermolabile variant of the methylenetetrahydrofolate reductase gene: a potential risk factor for hyperhomocysteinaemia, CVD, and stroke in childhood. Dev Med Child Neurol 2001; 43: 220–225.

Van Raay Y, Darteyre S, Di Rocco F, Goodden J, Papouin M, Brunelle F, Sainte-Rose C, Zérah M. Neonatal ruptured intracranial aneurysms: case report and literature review. Childs Nerv Syst 2009; 25: 1025–1033.

Rafay MF, Pontigon A-M, Chiang J, Adams M, Jarvis DA, Silver F, MacGregor D, deVeber GA. Delay to diagnosis in acute pediatric arterial ischemic stroke. Stroke 2009; 40: 58–64.

Rafay MF, Cortez MA, deVeber GA, Tan-Dy C, Al-Futaisi A, Yoon W, Fallah S, Moore AM. Predictive value of clinical and EEG features in the diagnosis of stroke and hypoxic ischemic encephalopathy in neonates with seizures. Stroke 2009; 40: 2402–2407.

Raju TNK, Nelson KB, Ferriero D, Lynch J. NICHD-NINDS Perinatal Stroke Workshop Participants. Ischaemic perinatal stroke: summary of a workshop sponsored by the National Institute of Child Health and Human Development and the National Institute of Neurological Disorders and Stroke. Pediatrics 2007; 120: 609–616.

Ramaswamy V, Miller SP, Barkovich AJ, Partrige JC, Ferriero DM. Perinatal stroke in term infants with neonatal encephalopathy. Neurology 2004; 62: 2088–2091.

Ramenghi LA, Govaert P, Fumagalli M, Bassi L, Mosca F. Neonatal cerebral sinovenous thrombosis. Semin Fetal Neonatal Medicine 2009; 14: 278–283.

Rasul CH, Mahboob AA, Hossain SM, Ahmed KU. Predisposing factors and outcome of stroke in childhood. Indian Pediatr 2009; 46: 419–421.
Rees DC. The population genetics of factor V Leiden (Arg 506 Gln). Br J Haematol 1996; 95: 579–586.
Renowden S. Cerebral venous sinus thrombosis. Eur Radiol 2004; 14: 215–226.
De Ribaupierre S, Rillet B, Cotting J, Regli L. A 10-year experience in paediatric spontaneurs cerebral hemorrhage: which children with headache need more than a clinical examination? Swiss Med Wkly 2008; 138: 59–69.
Roach ES, Golomb MR, Adams R, Biller J, Daniels S, deVeber G, Ferriero D, Jones BV, Kirkham FJ, Scott RM, Smith ER. Management of stroke in infants and children: A scientific statement from a special writing group of the American Heart Association Stroke Council and the Council on Cardiovascular Disease in the Young. Stroke. 2008; 39: 2644–2691.
Rosendaal FR, Doggen CJM, Zivelin A, Arruda VR, Aiach M, Siscovick DS, Hillarp A, Watzke HH, Bernardi F, Cumming AM, Preston FE, Reitsma PH. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost 1998; 79: 706–708.
Sachs BP, Acker D, Tuomala R, Brown E. The incidence of symptomatic intracranial hemorrhage in term appropriate-for-gestation-age infants. Clin Pediatr (Phila) 1987; 26: 355–358.
Sandberg DI, Lamberti-Pasculli M, Drake JM, Humphreys RP, Rutka JT. Spontaneous intraparenchymal hemorrhage in full-term neonates. Neurosurgery 2001; 48: 1042–1048.
Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress: a clinical and elctroencephalographic study. Arch Neurol 1976; 33: 696–705.
Schulzke S, Weber P, Luetschg J, Fahnenstich H. Incidence and diagnosis of unilateral arterial cerebral infarction in newborn infants. J Perinat Med 2005; 33: 170–175.
Schoenberg BS, Mellinger JF, Schoenberg DG. Cerebrovascular disease in infants and children: a study of incidence, clinical features, and survival. Neurology 1978; 28: 763–768.
Sébire G, Fullerton H, Riou E, deVeber G. Toward the definition of cerebral arteriopathies of childhood. Curr Opin Pediatr 2004; 16: 617–622.
Sébire G, Tabarki B, Saunders DE, Leroy I, Liesner R, Saint-Martin C, Husson B, Williams AN, Wade A, Kirkham FJ. Cerebral venous sinus thrombosis in children: risk factors, presentation, diagnosis and outcome. Brain 2005; 128: 477–489.
Seidman C, Kirkham F, Pavlakis S. Pediatric stroke: current developments. Curr Opin Pediatr 2007; 19: 657–662.
Shaffer L, Rich PM, Pohl KRE, Ganesan V. Can mild head injury cause ishaemic stroke? Arch Dis Child 2003; 88: 267–269.
Shellhaas RA, Smith SE, O'Tool E, Licht DJ and Ichord RN. Mimics of childhood stroke: characteristics of a prospective cohort. Pediatrics 2006; 118: 704–709.
Shi KL, Wang JJ, Li JW, Jiang LQ, Mix E, Fang F, Wu HS, Jin X, Jing H, Zou LP. Arterial ischemic stroke: Experience in Chinese children. Pediatr Neurol 2008; 38: 186–90.
Simchen MJ, Goldstein G, Lubetsky A, Strauss T, Schiff E, Kenet G. Factor V Leiden and antiphospholipid antibodies in either mothers or infants increase the risk for perinatal arterial ischemic stroke. Stroke 2009; 40: 65–70.
Simma B, G Martin, T Muller and M Huemer, Risk factors for pediatric stroke: consequences for therapy and quality of life. Pediatr Neurol 2007; 37: 121–126.

Sirachainan N, Tapanapruksakul P, Visudtibhan A, Chuansumrit A, Cheeramakara C, Atamasirikul K, Chotsuppakarn S, Areekul S. Homocysteine, MTHFR C677 T, vitamin B12, and foliate levels in Thai children with ischemic stroke: A case-control study. J Pediatr Hematol Oncol 2006; 28: 803–808.

Sreenan C, Bhargava R, Robertson CMT. Cerebral infarction in the term newborn: Clinical presentation and long-term outcome. J Pediatr 2000; 137: 351–355.

Srinivasan J, Miller SP, Phan TG, Mackay MT. Delayed recognition of initial stroke in children: need for increased awareness. Pediatrics 2009; 124: e227–234.

De Stefano V, Martinelli I, Mannucci PM, Paciaroni K, Chiusolo P, Casorelli I, Rossi E, Leone G. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med 1999; 341: 801–806.

Steinlin M, Pfister I, Pavlovic J, Everts R, Bolthauser E, Capone Mori A, Gubser Mercati D, Hänggeli CA, Keller E, Luetschg J, Marcoz J, Ramelli GP, Roulet Perez E, Schmitt-Mechelke T, Weissert M. The first three years of the Swiss Neuropaediatric Stroke Registry (SNPSR): A population-based study of incidence, symptoms and risk factors. Neuropediatrics 2005; 36: 90–97.

Stelmach T, Pisarev H, Talvik T. Ante- and perinatal factors for cerebral palsy: case-control study in Estonia. J Child Neurol 2005; 20: 654–660.

Sträter R, Vielhaber H, Kassenböhmer R, von Kries R, Göbel U, Nowak-Göttl U. Genetic risk factors of thrombophilia in ischaemic childhood stroke of cardiac origin. A prospective ESPED syrvey. Eur J Pediatr 1999; 158: S122–125.

Sträter R, Becker S, von Eckardstein A, Heinecke A, Gutsche S, Junker R, Kurnik K, Schobess R, Nowak-Göttl U. Prospective assessment of risk factors for recurrent stroke during childhood – a 5-year follow-up study. Lancet 2002; 360: 1540–1545.

Sudlow CLM, Warlow CP. Comparing stroke incidence worldwide. What makes the studies comparable? Stroke 1996; 27: 550–558.

Tadtaeva ZG, Katsadze YL. Hereditary thrombophilia: polymorphisms of some genes and hyperhomocysteinemia in pediatric patients with stroke. Insult 2007; 20: 50–54.

Talvik I, Metsvaht T, Leito K, Põder H, Kool P, Väli M, Lintrop M, Kolk A, Talvik T. Inflicted traumatic brain injury (ITBI) or shaken baby syndrome (SBS) in Estonia. Acta Paediatr 2006; 95: 799–804.

Talvik T, Tomberg T, Tolpats V, Lüüs SM, Toomela A, Soopõld T, Rätsep H, Kaasik AE A, Tammpere A. Kompjuterno-tomografičeskoe i kliničeskoe obsledovanie detej c gemiparezami. Žurnal Nevropatologii i Psihiatrii 1987; 3: 321–480.

Teksam M, Moharir M, deVeber G, Scroff M. Frequency and topographic distribution of brain lesions in pediatric cerebral venous thrombosis. Am J Neuroradiol 2008; 29: 1961–1965.

Trauner DA, Chase C, Walker P, Wulfeck B. Neurologic profiles of infants and children after perinatal stroke. Pediatr Neurol 1993; 9: 383–386.

deVeber G. Stroke and the child's brain: an overview of epidemiology, syndromes and risk factors. Curr Opin Neurol 2002; 15: 133–138.

deVeber G, Andrew M, Adams C, Bjorson B, Booth F, Buckley DJ, Camfield CS, David M, Humphreys P, Langevin P, MacDonald EA, Gillett J. Cerebral sinovenous thrombosis in children. N Engl J Med 2001; 345: 412–423.

Vibo R. The third stroke registry in Tartu, Estonia from 2001 to 2003: incidence, case-fatality, risk factors and long-term outcome. Dissertationes medicinae Universitatis Tartuensis. Tartu University Press, 2007. p.35.

Vizcaíno-Díaz C, Sánchez-Zaplana H, Ruiz JC, Jiménez-Cobo B. Rupture of intracranial arterial aneurysms in neonates: case report and review of the literature. J Child Neurol 2009; 24: 208–214.

Voetesch B, Jin RC, Bierl C, Benke KS, Kenet G, Simioni P, Ottaviano F, Damasceno BP, Annichino-Bizacchi JM, Handy D, Loscalzo J. Promoter polymorphisms in the plasma glutathione peroxidase (GPx-3) gene: a novel risk factor for AIS among young adults and children. Stroke 2007; 38: 41–49.

Volpe JJ. Intracranial hemorrhage: Germinal matrix-intraventricular hemorrhage. In: Neurology of the Newborn, 4th ed, Saunders Elsevier, Philadelphia 2001. p.406.

Volpe JJ. Intracranial hemorrhage: Subdural, primary subarachnoid, cerebellar, intraventricular, and miscellaneous. In: Neurology of the Newborn, 5th ed, Saunders Elsevier, Philadelphia 2008. p.507.

de Vries LS, Groenendaal F, Eken P, van Haastert IC, Rademarker KJ, Meiners LC. Infarcts in the vascular distribution of the middle cerebral artery in preterm and fullterm infants. Neuropediatrics 1997; 28: 88–96.

Wang JJ, Shi KL, Li JW, Jiang LQ, Caspi O, Fang F, Xiao J, Jing H, Zou LP. Risk factors for arterial ischemic and hemorrhagic stroke in childhood. Pediatric Neurology 2009; 40: 277–281.

Wasay M, Dai AI, Ansari M, Shaikh Z, Roach ES. Cerebral venous sinus thrombosis in children: a multicenter cohort from the United States. J Child Neurol 2008; 23: 26–31.

Wu YW, Miller SP, Chin K, Collins AE, Lomeli SC, Chuang NA, Barkovich AJ, Ferriero DM. Multiple risk factors in neonatal sinovenous thrombosis. Neurology 2002; 59: 438–440.

Wu YW, March WM, Croen LA, Grether JK, Escobar GJ, Newman TB. Perinatal stroke in children with motor impairment: A population-based study. Pediatrics 2004; 114: 612–619.

Wu YW, Lindan CE, Henning LH, Yoshida CK, Fullerton HJ, Ferriero DM, Barkovich AJ, Croen LA. Neuroimaging abnormalities in infants with congenital hemiparesis. Pediatr Neurol 2006; 35: 191–196.

Young G, Albisetti M, Bonduel M, Brandao l, Chan A, Fredrichs F, et al. Impact of inherited thrombophilia on venous thromboembolism in children: a systematic review and meta-analysis of observational studies. Circulation 2008; 118: 1373–1382.

Zahuranec DB, Brown DL, Lisabeth LD, Morgestern LB. Is it time for a large, collaborative study of pediatric stroke? Stroke 2005; 36: 1825–1829.

Zenz W, Bodó Z, Plotho J, Streif W, Male Ch, Bernert G, Rauter L, Ebetsberger G, Kaltenbrunner K, Kurnik P, Lischka A, Paky F, Ploier R, Höfler G, Mannhalter C, Muntean W. Factor V Leiden and prothrombin gene G 20210 A variant in children with ischemic stroke. Thromb Haemost 1998; 80: 763–766.

Zimmer JA, Garg BP, Williams LS, Golomb MR. Age-related variation in presenting signs of childhood arterial ischemic stroke. Pediatr Neurol 2007; 37: 171–175.

SUMMARY IN ESTONIAN

Laste insult Eestis – epidemioloogia ja riskitegurid

Lastel esineb insulti sagedamini, kui üldiselt arvatakse. Laste insuldi uuringute arv on viimastel aastakümnetel tunduvalt kasvanud. Peamised edusammud lapseea insuldi diagnoosimisel on seotud neuroradioloogiliste uurimisvõimaluste olulise paranemise, insuldi teket soodustavate kroonilisi haigusi põdevate laste elulemuse pikenemise ja arstkonna suurenenud teadlikkusega lapseea insuldist. Insulti esineb lastel täiskasvanutega võrreldes harvem ja kliiniline sümptomatoloogia on sageli mittespetsiifilisem (esinevad üldsümptomid: krambid, rahutus, oksendamine, teadvushäired). Laste insuldi tekkemehhanism jääb tihti ebaselgeks, sest arvestada tuleb paljusid erinevaid riskitegureid.

Insult lastel klassifitseeritakse lapse vanuse järgi insuldi tekkimisel: perinataalne insult (alates 20. rasedusnädalast kuni 28. elupäevani) ja lapseea insult (alates 1. elukuust kuni 18. eluaastani) (*Raju jt, 2007; Amlie-Lefond jt, 2008*). Olenevalt vaskulaarsest haaratusest jaguneb insult arteriaalseks ja venoosseks (sinovenoosne tromboos). Arteriaalne insult võib olla nii isheemiline kui ka hemorraagiline. Hemorraagilise insuldi osakaal lapseea insultidest on 30–40% (*Kleindorfer jt, 2006*), täiskasvanutel aga 17% (*Vibo, 2007*).

Perinataalse insuldi haigestumusmäär varieerub uuringutes 17 kuni 43 juhuni 100 000 elussünni kohta (*Wu jt, 2004; Schulzke jt, 2005*). Perinataalne insult jaotatakse diagnoosimise aja alusel neonataalseks insuldiks (diagnoositud vastsündinu perioodis) ja tõenäoliselt perinataalseks insuldiks (diagnoositud pärast 1. elukuud). Neonataalse insuldi korral esinevad vastsündinuperioodis kliinilised väljendunud sümptomid (krambid, teadvushäire jt), mis on näidustus aju piltdiagnostikaks. Tüüpiline leid neuroradioloogilistel uuringutel kinnitab neonataalse insuldi diagnoosi. Neonataalse insuldiga vastsündinud moodustavad 33–58% kõikidest perinataalse insuldiga lastest (*Wu jt, 2004; Lee jt, 2005*). Ülejäänud lastel vastsündinuperioodis sümptomeid ei esine või on tegemist minimaalsete sümptomitega, mistõttu piltdiagnostikat ei tehta ja lapsed satuvad arstide vastuvõtule mitmeid kuid hiljem hemipareesi ja/või krampide tõttu ning neuroradioloogilistel uuringutel ilmneb fokaalne vaskulaarse geneesiga vana kahjustuskolle. Sellistel juhtudel kasutatakse terminit ”tõenäoline perinataalne insult” (*Kirton jt, 2008; Golomb jt, 2008b*). Peamisteks perinataalse insuldi riskiteguriteks on ema infektsioonid ja trombofiilia, platsenta patoloogia, protrombootilised tegurid, südamerikked ja infektsioonid (*Lynch jt, 2009*).

Perinataalse insuldiga (neonataalne ja tõenäoline perinataalne insult) lastel ilmnevad hiljem mitmed tervisehäired, milleks on motoorikahäire, epilepsia ja kognitiivsed häired (*Lynch jt, 2009*). Neuroloogilistest probleemidest esinevad hilistulemusena sagedamini hemiparees (50–87%) (*Lee jt, 2005; Golomb jt, 2008b*) ja epilepsia (23–67%) (*Golomb jt, 2007; Golomb jt, 2001*). Perinataalse insuldi suremus ei ole teada, kuna diagnoos hilineb tihti ja surma põhjuseks on

sageli kaasuv haigus (*Lee jt, 2005*). Insuldi kordumisrisk pärast perinataalset insulti on väike (3%) (*Kurnik jt, 2003*).

Lapseea insuldi haigestumusmäärad varieeruvad erinevates uuringutes 1,3 kuni 13 juhuni 100 000 lapse kohta aastas (*Earley jt, 1998; Giroud jt, 1995*). Ka lapseeas esineb krampe insuldi esmase sümptomina sagedamini kui täiskasvanutel (*Chadehumbe jt, 2009*). Peamisteks riskiteguriteks arteriaalse isheemilise insuldi korral on arteriopaatia, südamepatoloogia, kaasasündinud või omandatud protrombootilised häired, infektsioon ja trauma (*Dlamini jt, 2009*). Ajusiseste hemorraagiate põhjusteks on arteriovenoossed malformatsioonid, hematoloogilised häired, ajukasvajad ja südamerikked (k.a südamekirurgia) (*Jordan jt 2007; Lo jt, 2008*). Lapseea arteriaalse isheemilise insuldi diagnoosimine hilineb sageli – harva (10%) diagnoositakse ajuinfarkt lastel esimese 3 tunni jooksul (*Gabis jt, 2002*).

Sinovenoosset tromboosi esineb nii looteeas (*Laurichesse jt, 2008*), enneaegsetel, (*Bassan jt, 2006*), ajalistel vastsündinutel kui ka vanematel lastel (*deVeber jt, 2001; Heller jt, 2003*). Viimased uuringud on näidanud, et enneaegsete ühepoolne periventrikulaarne hemorraagiline infarkt (endise klassifikatsiooni järgi IV astme vatsakesesisene hemorraagia) on venoosne infarkt (*Volpe, 2001*). Sinovenoosset tromboosi esineb 0,67 juhul 100 lapse kohta ja 43%-l juhtudest esineb see vastsündinu perioodis (*deVeber jt, 2001*). Sümptomid sinovenoossee tromboosi korral on lastel, sh vastsündinutel, sageli mittespetsiifilised ja seetõttu arvatakse, et sinovenoosne tromboos on lastel aladiagnoositud (*Sébire, 2005*). Sinovenoosse tromboosi peamisteks riskiteguriteks on pea- ja kaelapiirkonna infektsioonid, ägedad ja kroonilised süsteemsed haigused, protrombootilised tegurid ja aneemia (*deVeber jt, 2001*).

Protrombootilistest teguritest on laste insuldi korral kõige rohkem uuritud viienda hüübimisteguri Leideni mutatsiooni (FVL) ja teise hüübimisteguri mutatsiooni (PT 20210G>A). Siiani on juhtkontrolluuringute tulemused olnud vastuolulised: selle peamiseks põhjuseks on arvatavasti väikesed uurimisrühmad, mis tingivad laiad usaldusintervallide vahemikud (*Casas jt, 2005*). Kaks metaanalüüsi on näidanud, et nii FVL kui ka PT 20210G>A soodustavad laste arteriaalse isheemilise insuldi teket (*Chan jt, 2000; Juul jt, 2002*).

Eestis ja ka mujal Ida-Euroopas puudusid kuni praeguse uurimuseni laste ajuinsuldi epidemioloogiat käsitlevad uuringud.

Uurimuse eesmärgid

1. Uurida perinataalse ja lapseea insuldi haigestumust.
2. Uurida perinataalse ja lapseea insuldi kliinilisi sümptomeid.
3. Uurida perinataalse ja lapseea insuldi riskitegureid.
4. Uurida perinataalse insuldi neuroloogilist hilistulemust.
5. Uurida lapseea arteriaalse isheemilise insuldi diagnoosimise kiirust.
6. Uurida FVL ja PT 20210G>A mutatsioonide seost laste isheemilise insuldiga.

Uurimisrühmad ja -meetodid

Perinataalse insuldi uuring toimus tagasivaatavalt aastatel 1994–2002 ja edasivaatavalt aastal 2003. Rahvusvahelise haiguste klassifikatsiooni (RHK-9 ja RHK-10) koodide otsingu ja isiklike kontaktide abil selgitati välja kõik kaasasündinud hemipareesiga lapsed, kes olid sündinud Ida- või Lõuna-Eestis ning kellele oli diagnoos pandud kas Tartu Ülikooli Kliinikumi (TÜK) Lastekliinikus või Tallinna Lastehaiglas. Uuringusse kaasamise kriteeriumid olid järgmised: a) fokaalne ajukahjustus (infarkt, ajusisene või subarahnoidaalne hemorraagia) kompuuter- või magnetresonantstomograafia uuringul, b) gestatsioonivanus vähemalt 32 nädalat ja c) insult tekkinud perinataalses perioodis (kuni 1 kuu vanuses). Uuringust väljaarvamise kriteeriumiteks olid fokaalse ajukahjustuse mittevaskulaarsed põhjused: metaboolne insult, trauma, entsefaliit ja ajutuumor. Uuringukriteeriumitele vastas 38 last. Laste jälgimisaeg oli keskmiselt 7 aastat (2 kuni 12 aastat).

Lapseea insuldi epidemioloogiline uuring oli edasivaatav ja hõlmas kogu Eestit. Patsiendid leiti uuringusse 1) RHK-10 koodide otsinguga TÜK lastekliinikust, Tallinna Lastehaiglast, TÜK närvikliinikust, TÜK kardioloogiakliinikust ja Põhja-Eesti Regionaalhaiglast, 2) isiklike kontaktide põhjal, 3) TÜK kohtumeditsiini- ja lahanguprotokollidest. Uuringurühma kaasati kõik lapsed vanuses 1 kuu kuni 18 aastat, kes haigestusid insulti aastatel 2004–2006 (22 last). Kliiniliste sümptomite ja riskitegurite analüüsi olid kaasatud ka tagasivaatavalt tuvastatud aastatel 1995–2003 insulti haigestunud lapsed Ida- ja Lõuna-Eestist (kokku 48 last). Uuringusse kaasati lapsed, kellel oli diagnoositud transitoorne ajuisheemia, arteriaalne isheemiline insult, hemorraagiline insult või sinovenoosne tromboos.

Lapseea insuldi **diagnoosimise kiiruse** uuringus osalesid epidemioloogilises uuringus tuvastatud 22 arteriaalse isheemilise insuldiga last.

Geneetiliste riskitegurite juhtkontrolluuringus osales 75 perinataalse või lapseea insuldiga last kogu Eestist, kellele oli DNA-uuring tehtud aastatel 2004–2009. Kontrollrühmaks olid 2005. aasta jaanuaris järjestikku üle Eesti sündinud 400 vastsündinut, kelle testkaardi vereplekkidest eraldati DNA. FVL ja PT 20210G>A polümorfismid tuvastati samaaegselt. FVL ja PT 20210G>A seost laste sinovenoosse tromboosiga uuriti varem avaldatud ja Eesti laste juhtkontrolluuringute metaanalüüsi põhjal.

Uurimuse tulemused ja arutelu

Perinataalse insuldi haigestumus Eestis on 63 juhtu 100 000 ehk 1 juht 1578 elussünni kohta aastas, mis on suurem, kui mujal maailmas seni avaldatud andmed näitavad (vt tabel 1 lk 12). Üheks põhjuseks võib olla see, et erinevalt teistest avaldatud töödest olid Eesti laste uuringusse kaasatud ka hemorraagilise insuldiga patsiendid. Teise tegurina, mis mõjutab Eesti laste suurt haigestumust,

tuleb arvestada fakti, et uurimisrühma olid kaasatud ka tõenäolise perinataalse insuldiga lapsed, kes moodustasid 66% kogu perinataalse insuldi grupist.

Lapseea insuldi haigestumus Eestis on 2,7 uut juhtu 100 000 lapse kohta aastas. See tulemus on sarnane mujal tehtud uuringute tulemustega (vt tabel 2 lk 19). Lapseea sinovenoosse tromboosi haigestumus on 0,25 uut juhtu 100 000 lapse kohta aastas, mis on veidi väiksem kui varem publitseeritud, kuid sinovenoosne tromboos võib olla lastel aladiagnoositud. Eesti laste insuldi uuring on esimene, kuhu olid kaasatud lapsed transitoorse ajuisheemiaga. Uuringu andmetel oli transitoorse ajuisheemia haigestumusmäär 0,37 juhtu 100 000 lapse kohta aastas. Samas on võimalik, et haigestumus võib olla suurem, sest kiiresti mööduvate sümptomite tõttu ei pöördu vanemad arsti poole. Nii neonataalset kui ka lapseea insulti esines poistel rohkem kui tüdrukutel ning see tulemus on kooskõlas varasemate uuringutega.

Ühel kolmandikul perinataalse insuldiga patsientidest avaldusid insuldile viitavad sümptomid (krambid, teadvushäire) esimestel elupäevadel ja neuroradioloogilised uuringud kinnitasid insuldi diagnoosi (neonataalne insult). Ülejäänud kahel kolmandikul juhtudest pöördusid vanemad arsti poole keskmiselt 8 kuu vanuses (1 kuu kuni 2 aastat) peamiselt (85%) ühe kehapoole nõrkuse tõttu ja neuroradioloogilised uuringud kinnitasid insuldi diagnoosi (tõenäoline perinataalne insult).

Neuroloogiline koldeleid esines kõigil lapseea arteriaalse insuldiga lastel, 64%-l ajusisese hemorraagiaga lastest ja ühel kahest sinovenoosse tromboosiga lapsest.

Kõige sagedamini esinevateks perinataalse insuldi riskiteguriteks uurimisrühmas olid esmassünnitus, erakorraline keisrilõge, Apgari hinne 1. minutil < 7, preeklampsia ja protrombootililised tegurid. Seejuures esinesid esmassünnitus, suurem sünnikaal ja sünniasfüksia sagedamini neonataalse insuldiga lastel kui tõenäolise perinataalse insuldiga lastel ($p < 0,05$).

Riskitegurid olid tuvastatavad 85%-l lapseea insuldiga lastest. Arteriopaatia esines 25%-l, kardiaalne patoloogia 24%-l ja vähemalt üks protrombootiline tegur 39%-l uuritud ajuinfarktiga lastest. Arteriovenoosne malformatsioon leiti 59%-l intratserebraalse hemorraagiaga lastest. Ühelgi insuldiga lapsel ei olnud eelnenud kirurgilist vahelesegamist vajavat südamepatoloogiat ega ajutuumorit, mis on olnud sage teiste uurijate andmetel. Üheks seletuseks vaskulaarsete tüsistuste puudumise kohta kardiokirurgilistel ja onkoloogilistel pediaatrilistel patsientidel võib olla kardiokirurgia ja onkoloogia hea kvaliteet Eestis, teisalt on siiski võimalus, et mööduvad kerged neuroloogilised sümptomid jäävad tähelepanuta.

Kõigil perinataalse insuldiga lastel esines jääkleiuna hemiparees, sealhulgas mõõdukas või raske aste esines 76%-l. Epilepsia esines 33%-l lastest. Seejuures esines epilepsiat sama sagedusega nii neonataalse kui ka tõenäoliselt perinataalse insuldiga lastel.

Lapseea ajuinfarkti diagnoos hilines keskmiselt 9,2 päeva (mediaan 5,5 päeva). Esimese kuue tunni jooksul diagnoositi ajuinfarkt 18%-l lastest. Pea-

mine ajakadu esines alates esimesest neuroradioloogilisest uuringust (peamiselt kompuutertomograafia) kuni lõpliku diagnoosini, mis oli keskmiselt 4,3 päeva (mediaan 4,8 päeva). Üheks põhjuseks võib olla see, et ainult 41%-l juhtudest püstitati esialgse neuroradioloogilise uuringu alusel ajuinfarkti kahtlus. Laste ajuinfarkti diagnoosimist saab kiirendada, kasutades tänapäevaseid neuroradioloogilisi uuringuid (magnetresonantstomograafia tavalõiked, difusioonikujutised, magnetresonantsangiograafia) võimaluse korral esmavalikuna või lühikese aja jooksul pärast negatiivset kompuutertomograafia vastust. Lapseea ajuinfarkti varane diagnoosimine on oluline, kuna siis on võimalik rakendada hüperakuutset ravi (nt trombolüüsi), mis võib oluliselt vähendada neuroloogiliste jääknähtude esinemist ja nende raskust.

Tehtud juhtkontrolluuring näitas, et nii FVL (OR = 12,9; 95% CI 2,28–73,0) kui ka PT 20210G>A (OR = 11,9; 95% CI 2,11–67,2) on seotud sinovenoosse tromboosiga lastel. Laste arteriaalse isheemilise insuldiga seost ei leitud. Metaanalüüsid näitasid, et nii FVL kui ka PT 20210G>A suurendavad lastel riski haigestuda sinovenoossesse tromboosi 3,1 korda (CI 95% 1,8–5,5 FVL korral ja CI 95% 1,4–6,8 PT 20210G>A korral). Selgus, et laste uurimine sinovenoosse tromboosi korral FVL ja PT 20210G>A suhtes on vajalik.

Uurimuse järeldused

1. Perinataalse insuldi haigestumus Eestis on 63 juhtu 100 000 ehk 1 juht 1578 elussünni kohta – seda on rohkem kui varem mujal maailmas leitud. Lapseea insuldi esmashaigestumus Eestis on 2,73 juhtu 100 000 lapse kohta aastas, mis on sarnane teiste uuringute tulemustega.
2. Ühel kolmandikul perinataalse insuldiga lastest esinevad neuroloogilised sümptomid (krambid, teadvushäire) vastsündinuperioodis. Ülejäänud kaks kolmandikku lastest jõuab eriarsti vastuvõtule keskmiselt 8 kuu vanuses peamiselt (85%) hemipareesi tõttu. Neuroloogiline koldesümptomaatika esineb kõigil ajuinfarkti haigestunud lastel ja 64%-l ajusisese hemorragiaga lastest.
3. Riskitegurid leitakse enamusel (≥ 85%) perinataalse ja lapseea insuldiga lastest. Perinataalse insuldi riskiteguriteks olid sagedamini esmassünnitus, erakorraline keisrilõige, Apgari hinne 1. minutil < 7, preeklampsia ja protrombootilised tegurid. Arteriopaatia, südamepatoloogia ja protrombootilised tegurid esinesid sagedamini lapseea ajuinfarkti korral.
4. Kõigil perinataalse insuldiga lastel esines jääkleiuna hemiparees, mõõdukas või raske aste esines 76%-l lastest. Epilepsia esines ühel kolmandikul nii neonataalse kui ka tõenäoliselt perinataalse insuldiga lastest.
5. Lapseea ajuinfarkti diagnoos hilineb sageli. Suurim ajakadu esineb esmasest neuroradioloogilisest uuringust kuni lõpliku diagnoosini, kuna sageli on esmane neuroradioloogiline uuring negatiivse tulemusega.

6. FVL ja PT 20210G>A mutatsioonid suurendavad lastel sinovenoosse tromboosi riski 3 korda. Seega on laste uurimine sinovenoosse tromboosi korral FVL ja PT 20210G>A suhtes vajalik.

21

ACKNOWLEDGEMENTS

First of all, I wish to express my deepest gratitude to my supervisors: Professor Tiina Talvik for inspiring me to begin this study and for motivating, correcting and teaching me through the years, and Senior Research Fellow Dr Anneli Kolk for encouraging, teaching and correcting me during my work on this thesis. Both of my supervisors have taught me how to successfully share my love for my family and for science.

I would like to thank Professor Vallo Tillmann for his useful advice and for initiating the researchers' seminars, which generated ideas in the planning phase of the study.

I am very thankful to Associate Professor Dr Oivi Uibo and Senior Research Fellow Dr Janika Kõrv for their valuable suggestions and critical remarks in reviewing the thesis.

I am very thankful to all my co-authors for their valuable contribution. In particular, I would like to thank Dr Tiiu Tomberg and Dr Pilvi Ilves for their invaluable contributions as neuroradiologists and for the discussions we had about neuroradiology. I am very grateful to Dr Tiina Kahre for her enthusiasm and support in studying the genetic risk factors of paediatric stroke. I also gratefully acknowledge the assistance offered by Dr Tuuli Metsvaht in editing all the three manuscripts and for supporting me as an intensive care resident during the final preparation of the dissertation. I am very grateful to Ms Pille Kool for her valuable help with statistical analysis.

My warm thanks go to the child neurologists at the Children's Clinic of Tartu University Hospital – Inga Talvik, Eve Õiglane-Šlik, Reet Rein, Ulvi Vaher, Aita Napa and Reet Tali – whose continuing work with children with stroke made this study possible and who generously shared their knowledge with me. My special thanks go to co-workers from Tallinn Children's Hospital – Ülle Uustalu, Valentin Sander and Liis Toome – for taking their time to help me with my research work. In addition, I am thankful to all collegues at the Children's Clinic of Tartu University Hospital who provided information about the stroke patients they treated.

I am very thankful to Dr Tiina Vinni, a secretary at the department of paediatrics, for always solving my practical issues. I would like to thank Mrs Mare Saar, a statistician at the Children's Clinic of Tartu University Hospital, for always finding time to help me search medical cards from the archive and talk about life. I am very thankful to Ms Tuuli Ruus, a specialist of doctoral studies, for always being supportive and finding good solutions for every issue.

This study would never have been completed without the understanding and support of my family: my husband Allar and my daughters Marie and Liisa. I owe special thanks to my mother-in-law, Ellen Laugesaar, for her unselfish assistance in caring for my children. I am much obligated to my parents, Maris and Jüri Jürgenson, for my upbringing and for their prayers.

Special thanks are also due to the children and their families who participated in the study.

This study was partly financed by the Estonian Science Foundation (GARLA 6627 and GARLA 5462) and by the Estonian Ministry of Education and Research (TARLA 0475, TARLA 2695, and DARLA 3144).

PUBLICATIONS

CURRICULUM VITAE

Rael Laugesaar

Citizenship:	Estonian
Date and place of birth:	28 April 1979, Tartu
Address:	Lunini 6, 51014 Tartu
Phone:	+372 7 319 604
Fax:	+372 7 319 508
E-mail:	Rael.Laugesaar@kliinikum.ee

Education

1986–1994	Tartu 5th Secondary School
1994–1997	Hugo Treffner's Gymnasium
1997–2003	University of Tartu, Faculty of Medicine
2003–	University of Tartu, Faculty of Medicine, doctoral studies in paediatrics
2007–	University of Tartu, Faculty of Medicine, residency in paediatrics

Honours

2007	Participant at the national Estonian competition of undergraduate and postgraduate scientists. Diploma.
2007	Centre of Molecular and Clinical Medicine. Best article prize for young researchers.

Publications

A total of 10 publications: four in international journals, two in the journal *Eesti Arst* and four as conference papers.

1. Talvik T, Kolk A, Laugesaar R, Eelmäe I, Metsvaht T. Incidence of neonatal stroke in Estonia (Pilot Study). Stroke 2004; 35: E244.
2. Laugesaar R, Eelmäe I, Metsvaht T, Lintrop M, Tomberg T, Filippov M, Ventsel G, Kolk A, Talvik T. Vastsündinu insult – haigestumus Eestis. Eesti Arst 2004; 83: 296–303.
3. Laugesaar R, Tomberg T, Kolk A, Talvik T. Epilepsy in Children after Stroke. International Journal of Stroke 2006; 1(Suppl 1): 98.
4. Laugesaar R, Kolk A, Tomberg T, Metsvaht T, Lintrop M, Varendi H, Talvik T. Acutely and retrospectively diagnosed perinatal stroke: A population-based study. Stroke 2007; 38: 2234–2240.
5. Metsvaht T, Laugesaar R, Kolk A, Talvik T. Perinatal stroke with no obvious cause – Response to letter by Temesvari. Stroke 2008; 39, E36–E37.
6. Eelmäe I, Laugesaar R, Lintrop M, Õunap K, Kolk A. Cerebral venous sinus thrombosis in a preterm neonate due to inherited antitrombin III deficiency. Acta Pædiatrica 2008; 97(Suppl. 458): 18–19.
7. Kepler K, Tomberg T, Kolk A, Ilves P, Laugesaar R, Randver R. Kõnekeskuse reorganisatsioon perinataalse insuldi korral: haigusjuhu kirjeldus. Eesti Arst 2009; 88: 52−57.
8. Laugesaar R, Kolk A, Uustalu Ü, Talvik T. Diagnosis of childhood stroke is often delayed. Cerebrovascular Diseases 2009; (Suppl. 6), 68.
9. Laugesaar R, Kolk A, Uustalu Ü, Ilves P, Tomberg T, and Talvik I, Köbas K, Sander V, Talvik T. Epidemiology of childhood stroke in Estonia. Pediatric Neurology 2010; 42: 93–100.
10. Laugesaar R, Kahre T, Kolk A, Uustalu Ü, Kool P, Talvik T. Factor V Leiden and prothrombin 20210G>A mutation and paediatric ischemic stroke: a case-control study and two meta-analyses. Acta Paediatrica 2010, DOI:10.1111/j.1651–2227.2010.01784.x

CURRICULUM VITAE

Rael Laugesaar

Kodakondsus:	Eesti
Sünniaeg ja -koht:	28. aprill 1979, Tartu
Aadress:	Lunini 6, 51014 Tartu
Telefon:	+372 7 319 604
Fax:	+372 7 319 508
E-post:	Rael.Laugesaar@kliinikum.ee

Haridus

1986–1994	Tartu 5. Keskkool
1994–1997	Hugo Treffneri Gümnaasium
1997–2003	Tartu Ülikool, arstiteaduskond, arstiteaduse põhiõpe
2003–	Tartu Ülikool, arstiteaduskond, doktoriõpe
2007–	Tartu Ülikool, arstiteaduskond, residentuur

Tunnustused

2007	Eesti üliõpilaste teadustööde riiklik konkurss. Diplom.
2007	Molekulaarse ja kliinilise meditsiini tippkeskuse artiklikonkursi esikoht

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos.** The natural course of gastric ulcer in connection with chronic gastritis and *Helicobacter pylori*. Tartu, 1991.
2. **Mihkel Zilmer.** Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar.** Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik.** Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation). Tartu, 1992.
5. **Ants Peetsalu.** Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and *Helicobacter pylori*. Tartu, 1992.
6. **Marika Mikelsaar.** Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus.** Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar.** Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm.** On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross.** Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo.** Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik.** The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa.** Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk.** Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar.** Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi.** The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar.** Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang.** The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak.** Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.
20. **Tõnis Karki.** Quantitative composition of the human lactoflora and method for its examination. Tartu, 1996.

21. **Reet Mändar.** Vaginal microflora during pregnancy and its transmission to newborn. Tartu, 1996.
22. **Triin Remmel.** Primary biliary cirrhosis in Estonia: epidemiology, clinical characterization and prognostication of the course of the disease. Tartu, 1996.
23. **Toomas Kivastik.** Mechanisms of drug addiction: focus on positive reinforcing properties of morphine. Tartu, 1996.
24. **Paavo Pokk.** Stress due to sleep deprivation: focus on $GABA_A$ receptor-chloride ionophore complex. Tartu, 1996.
25. **Kristina Allikmets.** Renin system activity in essential hypertension. Associations with atherothrombogenic cardiovascular risk factors and with the efficacy of calcium antagonist treatment. Tartu, 1996.
26. **Triin Parik.** Oxidative stress in essential hypertension: Associations with metabolic disturbances and the effects of calcium antagonist treatment. Tartu, 1996.
27. **Svetlana Päi.** Factors promoting heterogeneity of the course of rheumatoid arthritis. Tartu, 1997.
28. **Maarike Sallo.** Studies on habitual physical activity and aerobic fitness in 4 to 10 years old children. Tartu, 1997.
29. **Paul Naaber.** *Clostridium difficile* infection and intestinal microbial ecology. Tartu, 1997.
30. **Rein Pähkla.** Studies in pinoline pharmacology. Tartu, 1997.
31. **Andrus Juhan Voitk.** Outpatient laparoscopic cholecystectomy. Tartu, 1997.
32. **Joel Starkopf.** Oxidative stress and ischaemia-reperfusion of the heart. Tartu, 1997.
33. **Janika Kõrv.** Incidence, case-fatality and outcome of stroke. Tartu, 1998.
34. **Ülla Linnamägi.** Changes in local cerebral blood flow and lipid peroxidation following lead exposure in experiment. Tartu, 1998.
35. **Ave Minajeva.** Sarcoplasmic reticulum function: comparison of atrial and ventricular myocardium. Tartu, 1998.
36. **Oleg Milenin.** Reconstruction of cervical part of esophagus by revascularised ileal autografts in dogs. A new complex multistage method. Tartu, 1998.
37. **Sergei Pakriev.** Prevalence of depression, harmful use of alcohol and alcohol dependence among rural population in Udmurtia. Tartu, 1998.
38. **Allen Kaasik.** Thyroid hormone control over β-adrenergic signalling system in rat atria. Tartu, 1998.
39. **Vallo Matto.** Pharmacological studies on anxiogenic and antiaggressive properties of antidepressants. Tartu, 1998.
40. **Maire Vasar.** Allergic diseases and bronchial hyperreactivity in Estonian children in relation to environmental influences. Tartu, 1998.
41. **Kaja Julge.** Humoral immune responses to allergens in early childhood. Tartu, 1998.
42. **Heli Grünberg.** The cardiovascular risk of Estonian schoolchildren. A cross-sectional study of 9-, 12- and 15-year-old children. Tartu, 1998.

43. **Epp Sepp.** Formation of intestinal microbial ecosystem in children. Tartu, 1998.
44. **Mai Ots.** Characteristics of the progression of human and experimental glomerulopathies. Tartu, 1998.
45. **Tiina Ristimäe.** Heart rate variability in patients with coronary artery disease. Tartu, 1998.
46. **Leho Kõiv.** Reaction of the sympatho-adrenal and hypothalamo-pituitary-adrenocortical system in the acute stage of head injury. Tartu, 1998.
47. **Bela Adojaan.** Immune and genetic factors of childhood onset IDDM in Estonia. An epidemiological study. Tartu, 1999.
48. **Jakov Shlik.** Psychophysiological effects of cholecystokinin in humans. Tartu, 1999.
49. **Kai Kisand.** Autoantibodies against dehydrogenases of α-ketoacids. Tartu, 1999.
50. **Toomas Marandi.** Drug treatment of depression in Estonia. Tartu, 1999.
51. **Ants Kask.** Behavioural studies on neuropeptide Y. Tartu, 1999.
52. **Ello-Rahel Karelson.** Modulation of adenylate cyclase activity in the rat hippocampus by neuropeptide galanin and its chimeric analogs. Tartu, 1999.
53. **Tanel Laisaar.** Treatment of pleural empyema — special reference to intrapleural therapy with streptokinase and surgical treatment modalities. Tartu, 1999.
54. **Eve Pihl.** Cardiovascular risk factors in middle-aged former athletes. Tartu, 1999.
55. **Katrin Õunap.** Phenylketonuria in Estonia: incidence, newborn screening, diagnosis, clinical characterization and genotype/phenotype correlation. Tartu, 1999.
56. **Siiri Kõljalg.** *Acinetobacter* – an important nosocomial pathogen. Tartu, 1999.
57. **Helle Karro.** Reproductive health and pregnancy outcome in Estonia: association with different factors. Tartu, 1999.
58. **Heili Varendi.** Behavioral effects observed in human newborns during exposure to naturally occurring odors. Tartu, 1999.
59. **Anneli Beilmann.** Epidemiology of epilepsy in children and adolescents in Estonia. Prevalence, incidence, and clinical characteristics. Tartu, 1999.
60. **Vallo Volke.** Pharmacological and biochemical studies on nitric oxide in the regulation of behaviour. Tartu, 1999.
61. **Pilvi Ilves.** Hypoxic-ischaemic encephalopathy in asphyxiated term infants. A prospective clinical, biochemical, ultrasonographical study. Tartu, 1999.
62. **Anti Kalda.** Oxygen-glucose deprivation-induced neuronal death and its pharmacological prevention in cerebellar granule cells. Tartu, 1999.
63. **Eve-Irene Lepist.** Oral peptide prodrugs – studies on stability and absorption. Tartu, 2000.
64. **Jana Kivastik.** Lung function in Estonian schoolchildren: relationship with anthropometric indices and respiratory symptomas, reference values for dynamic spirometry. Tartu, 2000.

65. **Karin Kull.** Inflammatory bowel disease: an immunogenetic study. Tartu, 2000.
66. **Kaire Innos.** Epidemiological resources in Estonia: data sources, their quality and feasibility of cohort studies. Tartu, 2000.
67. **Tamara Vorobjova.** Immune response to *Helicobacter pylori* and its association with dynamics of chronic gastritis and epithelial cell turnover in antrum and corpus. Tartu, 2001.
68. **Ruth Kalda.** Structure and outcome of family practice quality in the changing health care system of Estonia. Tartu, 2001.
69. **Annika Krüüner.** *Mycobacterium tuberculosis* – spread and drug resistance in Estonia. Tartu, 2001.
70. **Marlit Veldi.** Obstructive Sleep Apnoea: Computerized Endopharyngeal Myotonometry of the Soft Palate and Lingual Musculature. Tartu, 2001.
71. **Anneli Uusküla.** Epidemiology of sexually transmitted diseases in Estonia in 1990–2000. Tartu, 2001.
72. **Ade Kallas.** Characterization of antibodies to coagulation factor VIII. Tartu, 2002.
73. **Heidi Annuk.** Selection of medicinal plants and intestinal lactobacilli as antimicrobil components for functional foods. Tartu, 2002.
74. **Aet Lukmann**. Early rehabilitation of patients with ischaemic heart disease after surgical revascularization of the myocardium: assessment of health-related quality of life, cardiopulmonary reserve and oxidative stress. A clinical study. Tartu, 2002.
75. **Maigi Eisen.** Pathogenesis of Contact Dermatitis: participation of Oxidative Stress. A clinical – biochemical study. Tartu, 2002.
76. **Piret Hussar.** Histology of the post-traumatic bone repair in rats. Elaboration and use of a new standardized experimental model – bicortical perforation of tibia compared to internal fracture and resection osteotomy. Tartu, 2002.
77. **Tõnu Rätsep.** Aneurysmal subarachnoid haemorrhage: Noninvasive monitoring of cerebral haemodynamics. Tartu, 2002.
78. **Marju Herodes.** Quality of life of people with epilepsy in Estonia. Tartu, 2003.
79. **Katre Maasalu.** Changes in bone quality due to age and genetic disorders and their clinical expressions in Estonia. Tartu, 2003.
80. **Toomas Sillakivi.** Perforated peptic ulcer in Estonia: epidemiology, risk factors and relations with *Helicobacter pylori*. Tartu, 2003.
81. **Leena Puksa.** Late responses in motor nerve conduction studies. F and A waves in normal subjects and patients with neuropathies. Tartu, 2003.
82. **Krista Lõivukene**. *Helicobacter pylori* in gastric microbial ecology and its antimicrobial susceptibility pattern. Tartu, 2003.
83. **Helgi Kolk.** Dyspepsia and *Helicobacter pylori* infection: the diagnostic value of symptoms, treatment and follow-up of patients referred for upper gastrointestinal endoscopy by family physicians. Tartu, 2003.

84. **Helena Soomer.** Validation of identification and age estimation methods in forensic odontology. Tartu, 2003.
85. **Kersti Oselin.** Studies on the human MDR1, MRP1, and MRP2 ABC transporters: functional relevance of the genetic polymorphisms in the *MDR1* and *MRP1* gene. Tartu, 2003.
86. **Jaan Soplepmann.** Peptic ulcer haemorrhage in Estonia: epidemiology, prognostic factors, treatment and outcome. Tartu, 2003.
87. **Margot Peetsalu.** Long-term follow-up after vagotomy in duodenal ulcer disease: recurrent ulcer, changes in the function, morphology and *Helicobacter pylori* colonisation of the gastric mucosa. Tartu, 2003.
88. **Kersti Klaamas.** Humoral immune response to *Helicobacter pylori* a study of host-dependent and microbial factors. Tartu, 2003.
89. **Pille Taba.** Epidemiology of Parkinson's disease in Tartu, Estonia. Prevalence, incidence, clinical characteristics, and pharmacoepidemiology. Tartu, 2003.
90. **Alar Veraksitš**. Characterization of behavioural and biochemical phenotype of cholecystokinin-2 receptor deficient mice: changes in the function of the dopamine and endopioidergic system. Tartu, 2003.
91. **Ingrid Kalev.** CC-chemokine receptor 5 (CCR5) gene polymorphism in Estonians and in patients with Type I and Type II diabetes mellitus. Tartu, 2003.
92. **Lumme Kadaja.** Molecular approach to the regulation of mitochondrial function in oxidative muscle cells. Tartu, 2003.
93. **Aive Liigant**. Epidemiology of primary central nervous system tumours in Estonia from 1986 to 1996. Clinical characteristics, incidence, survival and prognostic factors. Tartu, 2004.
94. **Andres, Kulla.** Molecular characteristics of mesenchymal stroma in human astrocytic gliomas. Tartu, 2004.
95. **Mari Järvelaid.** Health damaging risk behaviours in adolescence. Tartu, 2004.
96. **Ülle Pechter.** Progression prevention strategies in chronic renal failure and hypertension. An experimental and clinical study. Tartu, 2004.
97. **Gunnar Tasa.** Polymorphic glutathione S-transferases – biology and role in modifying genetic susceptibility to senile cataract and primary open angle glaucoma. Tartu, 2004.
98. **Tuuli Käämbre.** Intracellular energetic unit: structural and functional aspects. Tartu, 2004.
99. **Vitali Vassiljev.** Influence of nitric oxide syntase inhibitors on the effects of ethanol after acute and chronic ethanol administration and withdrawal. Tartu, 2004.
100. **Aune Rehema.** Assessment of nonhaem ferrous iron and glutathione redox ratio as markers of pathogeneticity of oxidative stress in different clinical groups. Tartu, 2004.
101. **Evelin Seppet.** Interaction of mitochondria and ATPases in oxidative muscle cells in normal and pathological conditions. Tartu, 2004.

102. **Eduard Maron.** Serotonin function in panic disorder: from clinical experiments to brain imaging and genetics. Tartu, 2004.
103. **Marje Oona.** *Helicobacter pylori* infection in children: epidemiological and therapeutic aspects. Tartu, 2004.
104. **Kersti Kokk.** Regulation of active and passive molecular transport in the testis. Tartu, 2005.
105. **Vladimir Järv.** Cross-sectional imaging for pretreatment evaluation and follow-up of pelvic malignant tumours. Tartu, 2005.
106. **Andre Õun.** Epidemiology of adult epilepsy in Tartu, Estonia. Incidence, prevalence and medical treatment. Tartu, 2005.
107. **Piibe Muda.** Homocysteine and hypertension: associations between homocysteine and essential hypertension in treated and untreated hypertensive patients with and without coronary artery disease. Tartu, 2005.
108. **Külli Kingo.** The interleukin-10 family cytokines gene polymorphisms in plaque psoriasis. Tartu, 2005.
109. **Mati Merila.** Anatomy and clinical relevance of the glenohumeral joint capsule and ligaments. Tartu, 2005.
110. **Epp Songisepp**. Evaluation of technological and functional properties of the new probiotic *Lactobacillus fermentum* ME-3. Tartu, 2005.
111. **Tiia Ainla.** Acute myocardial infarction in Estonia: clinical characteristics, management and outcome. Tartu, 2005.
112. **Andres Sell.** Determining the minimum local anaesthetic requirements for hip replacement surgery under spinal anaesthesia – a study employing a spinal catheter. Tartu, 2005.
113. **Tiia Tamme.** Epidemiology of odontogenic tumours in Estonia. Pathogenesis and clinical behaviour of ameloblastoma. Tartu, 2005.
114. **Triine Annus**. Allergy in Estonian schoolchildren: time trends and characteristics. Tartu, 2005.
115. **Tiia Voor.** Microorganisms in infancy and development of allergy: comparison of Estonian and Swedish children. Tartu, 2005.
116. **Priit Kasenõmm.** Indicators for tonsillectomy in adults with recurrent tonsillitis – clinical, microbiological and pathomorphological investigations. Tartu, 2005.
117. **Eva Zusinaite.** Hepatitis C virus: genotype identification and interactions between viral proteases. Tartu, 2005.
118. **Piret Kõll.** Oral lactoflora in chronic periodontitis and periodontal health. Tartu, 2006.
119. **Tiina Stelmach.** Epidemiology of cerebral palsy and unfavourable neurodevelopmental outcome in child population of Tartu city and county, Estonia Prevalence, clinical features and risk factors. Tartu, 2006.
120. **Katrin Pudersell.** Tropane alkaloid production and riboflavine excretion in the field and tissue cultures of henbane (*Hyoscyamus niger* L.). Tartu, 2006.
121. **Külli Jaako.** Studies on the role of neurogenesis in brain plasticity. Tartu, 2006.

122. **Aare Märtson.** Lower limb lengthening: experimental studies of bone regeneration and long-term clinical results. Tartu, 2006.
123. **Heli Tähepõld.** Patient consultation in family medicine. Tartu, 2006.
124. **Stanislav Liskmann.** Peri-implant disease: pathogenesis, diagnosis and treatment in view of both inflammation and oxidative stress profiling. Tartu, 2006.
125. **Ruth Rudissaar.** Neuropharmacology of atypical antipsychotics and an animal model of psychosis. Tartu, 2006.
126. **Helena Andreson.** Diversity of *Helicobacter pylori* genotypes in Estonian patients with chronic inflammatory gastric diseases. Tartu, 2006.
127. **Katrin Pruus.** Mechanism of action of antidepressants: aspects of serotoninergic system and its interaction with glutamate. Tartu, 2006.
128. **Priit Põder.** Clinical and experimental investigation: relationship of ischaemia/reperfusion injury with oxidative stress in abdominal aortic aneurysm repair and in extracranial brain artery endarterectomy and possibilities of protection against ischaemia using a glutathione analogue in a rat model of global brain ischaemia. Tartu, 2006.
129. **Marika Tammaru.** Patient-reported outcome measurement in rheumatoid arthritis. Tartu, 2006.
130. **Tiia Reimand.** Down syndrome in Estonia. Tartu, 2006.
131. **Diva Eensoo.** Risk-taking in traffic and Markers of Risk-Taking Behaviour in Schoolchildren and Car Drivers. Tartu, 2007.
132. **Riina Vibo.** The third stroke registry in Tartu, Estonia from 2001 to 2003: incidence, case-fatality, risk factors and long-term outcome. Tartu, 2007.
133. **Chris Pruunsild.** Juvenile idiopathic arthritis in children in Estonia. Tartu, 2007.
134. **Eve Õiglane-Šlik.** Angelman and Prader-Willi syndromes in Estonia. Tartu, 2007.
135. **Kadri Haller.** Antibodies to follicle stimulating hormone. Significance in female infertility. Tartu, 2007.
136. **Pille Ööpik.** Management of depression in family medicine. Tartu, 2007.
137. **Jaak Kals.** Endothelial function and arterial stiffness in patients with atherosclerosis and in healthy subjects. Tartu, 2007.
138. **Priit Kampus.** Impact of inflammation, oxidative stress and age on arterial stiffness and carotid artery intima-media thickness. Tartu, 2007.
139. **Margus Punab.** Male fertility and its risk factors in Estonia. Tartu, 2007.
140. **Alar Toom**. Heterotopic ossification after total hip arthroplasty: clinical and pathogenetic investigation. Tartu, 2007.
141. **Lea Pehme.** Epidemiology of tuberculosis in Estonia 1991–2003 with special regard to extrapulmonary tuberculosis and delay in diagnosis of pulmonary tuberculosis. Tartu, 2007.
142. **Juri Karjagin.** The pharmacokinetics of metronidazole and meropenem in septic shock. Tartu, 2007.
143. **Inga Talvik.** Inflicted traumatic brain injury shaken baby syndrome in Estonia – epidemiology and outcome. Tartu, 2007.

144. **Tarvo Rajasalu.** Autoimmune diabetes: an immunological study of type 1 diabetes in humans and in a model of experimental diabetes (in RIP-B7.1 mice). Tartu, 2007.
145. **Inga Karu.** Ischaemia-reperfusion injury of the heart during coronary surgery: a clinical study investigating the effect of hyperoxia. Tartu, 2007.
146. **Peeter Padrik.** Renal cell carcinoma: Changes in natural history and treatment of metastatic disease. Tartu, 2007.
147. **Neve Vendt.** Iron deficiency and iron deficiency anaemia in infants aged 9 to 12 months in Estonia. Tartu, 2008.
148. **Lenne-Triin Heidmets.** The effects of neurotoxins on brain plasticity: focus on neural Cell Adhesion Molecule. Tartu, 2008.
149. **Paul Korrovits.** Asymptomatic inflammatory prostatitis: prevalence, etiological factors, diagnostic tools. Tartu, 2008.
150. **Annika Reintam.** Gastrointestinal failure in intensive care patients. Tartu, 2008.
151. **Kristiina Roots.** Cationic regulation of Na-pump in the normal, Alzheimer's and CCK_2 receptor-deficient brain. Tartu, 2008.
152. **Helen Puusepp.** The genetic causes of mental retardation in Estonia: fragile X syndrome and creatine transporter defect. Tartu, 2009.
153. **Kristiina Rull.** Human chorionic gonadotropin beta genes and recurrent miscarriage: expression and variation study. Tartu, 2009.
154. **Margus Eimre.** Organization of energy transfer and feedback regulation in oxidative muscle cells. Tartu, 2009.
155. **Maire Link.** Transcription factors FoxP3 and AIRE: autoantibody associations. Tartu, 2009.
156. **Kai Haldre.** Sexual health and behaviour of young women in Estonia. Tartu, 2009.
157. **Kaur Liivak.** Classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Estonia: incidence, genotype and phenotype with special attention to short-term growth and 24-hour blood pressure. Tartu, 2009.
158. **Kersti Ehrlich.** Antioxidative glutathione analogues (UPF peptides) – molecular design, structure-activity relationships and testing the protective properties. Tartu, 2009.
159. **Anneli Rätsep.** Type 2 diabetes care in family medicine. Tartu, 2009.
160. **Silver Türk.** Etiopathogenetic aspects of chronic prostatitis: role of mycoplasmas, coryneform bacteria and oxidative stress. Tartu, 2009.
161. **Kaire Heilman.** Risk markers for cardiovascular disease and low bone mineral density in children with type 1 diabetes. Tartu, 2009.
162. **Kristi Rüütel.** HIV-epidemic in Estonia: injecting drug use and quality of life of people living with HIV. Tartu, 2009.
163. **Triin Eller.** Immune markers in major depression and in antidepressive treatment. Tartu, 2009.

164. **Siim Suutre.** The role of TGF-β isoforms and osteoprogenitor cells in the pathogenesis of heterotopic ossification. An experimental and clinical study of hip arthroplasty. Tartu, 2010.
165. **Kai Kliiman.** Highly drug-resistant tuberculosis in Estonia: Risk factors and predictors of poor treatment outcome. Tartu, 2010.
166. **Inga Villa.** Cardiovascular health-related nutrition, physical activity and fitness in Estonia. Tartu, 2010.
167. **Tõnis Org.** Molecular function of the first PHD finger domain of Autoimmune Regulator protein. Tartu, 2010.
168. **Tuuli Metsvaht.** Optimal antibacterial therapy of neonates at risk of early onset sepsis. Tartu, 2010.
169. **Jaanus Kahu.** Kidney transplantation: Studies on donor risk factors and mycophenolate mofetil. Tartu, 2010.
170. **Koit Reimand.** Autoimmunity in reproductive failure: A study on associated autoantibodies and autoantigens. Tartu, 2010.
171. **Mart Kull.** Impact of vitamin D and hypolactasia on bone mineral density: a population based study in Estonia. Tartu, 2010.