TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 863

MEDICA - ODONTOLOGICA

ANTENATAL INFLAMMATION AND BRAIN PATHOLOGY IN PRETERM INFANTS

by

Milla Reiman

TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2009

From the Department of Pediatrics, Turku University Hospital, University of Turku, Turku, Finland

Supervised by

Docent Liisa Lehtonen, MD
Department of Pediatrics
University of Turku
Turku, Finland

and

Docent Leena Haataja, MD
Department of Pediatric Neurology
University of Turku
Turku, Finland

Rewied by

Professor Seppo Heinonen, MD
Department of Obstetrics and Gynaecology
University of Kuopio
Kuopio, Finland

and

Docent Martin Renlund, MD
Department of Pediatrics
University of Helsinki
Helsinki, Finland

Opponent

Professor Mikko Hallman, MD
Department of Pediatrics
University of Oulu
Oulu, Finland

ISBN 978-951-29-3985-5 (PRINT)
ISBN 978-951-29-3986-2 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2009

To my Family

ABSTRACT

Milla Reiman. Antenatal inflammation and brain pathology in preterm infants. Department of Pediatrics, University of Turku.

Chorioamnionitis is known to be an important risk factor underlying preterm delivery, and it has also been suggested to associate with brain lesions and deviant neurological development in both preterm and term infants. Cytokines are believed to be the link causing the deleterious effects of inflammation to the nervous system. Their genetic regulation has also been suggested to play a role, as interleukin (IL)-6 -174 and -572 genotypes, which partly regulate IL-6 synthesis responses, have been connected with deviant neurological development in preterm infants. We evaluated the association of histological chorioamnionitis with brain lesions, regional brain volumes, and the functioning of the auditory pathway in very low birth weight/very low gestational age (VLBW/VLGA) infants. In addition, we investigated the association between IL-6 -174 and -572 genotypes and histological chorioamnionitis, neonatal infections, and brain lesions and regional brain volumes in VLBW/VLGA infants.

This study is a part of a larger multidisciplinary project PIPARI (Development and Functioning of Very Low Birth Weight Infants from Infancy to School Age), in which the survivors of a 6-year cohort of VLBW/VLGA infants (n=274) are being followed until school age in Turku University Central Hospital, Finland. Placental samples were collected in the delivery room, and were analyzed for histological inflammatory findings. Blood samples from the infants were collected and DNA was genotyped for IL-6-174 and -572 polymorphisms (GG/GC/CC). Brain ultrasound examinations were performed repeatedly in the neonatal intensive care unit and at term age, and were analysed for structural brain lesions. Brain magnetic resonance imaging was performed at term age, and was analysed for regional brain volumes. In addition, diffusion tensor imaging was performed at term, and was used to analyse fractional anisotrophy and the apparent diffusion coefficient of inferior colliculus. The brainstem auditory evoked potential recordings were carried out according to the routine clinical procedure at median age of 30 days after term age.

In our study, we found that histological chorioamnionitis was not an independent risk factor for brain lesions, reduced regional brain volumes or abnormal functioning of the auditory pathway in VLBW/VLGA infants. In addition, we found that IL-6 -174 GG and -572 GC genotypes were associated with a higher incidence of histological chorioamnionitis, and that -174 CC genotype associated with higher incidence of septicaemia. The analysed IL-6 genotypes were not associated with other brain lesions, but a reduced volume of basal ganglia and thalami was associated with IL-6 -174 CC and -572 GG genotypes.

In conclusion, our findings suggest that histological chorioamnionitis is not an independent risk factor for the brain development of VLBW/VLGA infants, or that the risk caused by inflammation does not exceed the risks attributed to other underlying pathologies behind preterm deliveries. In addition, our findings give reason to propose that IL-6 promoter genotypes have a role in the defence against serious infections and in the brain development of VLBW/VLGA infants.

Key Words: Preterm infant; Chorioamnonitis; Brain lesions; Brain volumes; Cytokines; Interleukin-6; Magnetic resonance imaging; Diffusion tensor imaging; Brainstem auditory evoked potentials

TIIVISTELMÄ

Milla Reiman. Antenataalinen inflammaatio ja pikkukeskosen aivolöydökset.
Lastenklinikka, Turun Yliopisto.

Istukan tulehdus eli korionamnoniitti on ennenaikaisen synnytyksen merkittävä riskitekijä, ja lisäksi korioamnioniitin on ajateltu lisäävän sekä keskosten että täysiaikaisena syntyneiden lasten aivokomplikaatioita ja neurologisen kehityksen ongelmia. Keskushermostovaikutu sten on ajateltu välittyvän sytokiinien kautta. Sytokiinien geneettisen säätelyn on esitetty olevan yhteydessä tulehduksen aiheuttamiin keskushermostovaikutuksiin, sillä interleukiini (IL)-6 geenin säätelyalueen -174 ja -572 genotyyppien on osoitettu olevan yhteydessä keskosten aivolöydöksiin ja heikompaan neurologiseen kehitykseen. Tämän väitöskirjatyön tarkoituksena oli tutkia histologisen korionamnioniitin yhteyttä pikkukeskosen aivolöydöksiin, aivojen osatilavuuksiin sekä kuuloradaston toimintaan. Lisäksi tutkimme IL-6 -174 ja -572 genotyyppien yhteyttä histologiseen korionamnioniittiin, vastasyntyneisyysajan infektioihin sekä aivolöydöksiin ja aivojen osatilavuuksiin pikkukeskosilla.

Tämä tutkimus on osa laajempaa kokonaisuutta (Pienipainoisten riskilasten käyttäytyminen ja kehitys imeväisiästä kouluikään, PIPARI), jossa kuuden vuoden kohorttia (lapsia n=274) seurataan kouluikään saakka. Tutkimukseen kutsuttiin Turun yliopistollisessa keskussairaalassa 1/2001-12/2006 välillä syntyneet alle 1501 g painavat keskoset, sekä huhtikuun 2004 jälkeen lisäksi kaikki alle 32 raskausviikolla syntyneet keskoset. Tutkimuslapsista kerättiin taustatietoja kyselylomakkeilla sekä äidin ja lapsen potilaskertomuksista. Tutkimuslasten istukat analysoitiin tulehdusmuutosten selvittämiseksi. Lasten verinäytteet kerättiin IL-6 genotyyppien selvittämiseksi (GG/GC/CC). Aivot kuvannettiin toistuvilla ultraäänitutkimuksilla sairaalahoidon aikana ja lasketussa ajassa, jolloin tehtiin myös aivojen magneettikuvaus (MRI). MRI-kuvista analysoitiin aivojen osatilavuudet. Lisäksi aivojen diffuusiotensorikuvaus tehtiin lasketussa ajassa, ja tästä määritettiin colliculus inferiorin fraktionaalinen anisotropia sekä diffuusiovakio. Aivorungon herätevastetutkimus tehtiin kliinisen seurannan mukaisesti mediaani-iässä 30 vuorokautta lasketun ajan jälkeen.

Tutkimuksessa todettiin, että histologinen korionamnioniitti ei lisännyt poikkeavia aivolöydöksiä eikä vaikuttanut aivojen osatilavuuksiin eikä kuuloradaston toimintaan pikkukeskosilla. Lisäksi selvitimme, että IL-6 -174 GG ja -572 GC genotyypit olivat yhteydessä histologisen korionamnioniitin suurempaan riskiin sekä IL-6 -174 CC genotyyppi oli yhteydessä sepsiksen suurempaan riskiin. Tutkitut IL-6 genotyypit eivät olleet yhteydessä poikkeaviin aivolöydöksiin, mutta basaaliganglioiden ja talamuksen pienentynyt tilavuus oli yhteydessä sekä IL-6 174 CC genotyyppiin että -572 GG genotyyppiin.

Löydöstemme perusteella histologinen korionamnioniitti ei ole itsenäinen riskitekijä pikkukeskosen poikkeavalle aivojen kehitykselle. Tulokset eivät poissulje histologisen korionamnioniitin haitallista vaikutusta keskosen aivojen kehitykselle, mutta riski ei vaikuttaisi olevan suurempi kuin muiden keskosuuteen liittyvien taustatekijöiden aiheuttama riski. Löydöksemme viittaavat siihen, että IL-6 säätelyalueen polymorfiat saattavat vaikuttaa sekä puolustuskykyyn vakavia infektioita vastaan että pikkukeskosen aivojen kehitykseen.

Avainsanat: Keskonen; Korionamnioniitti; Aivolöydökset; Aivojen osatilavuudet; Sytokiinit; Interleukiini-6; Magneettitutkimus; Diffuusiotensori kuvaus; Aivorungon herätevaste-tutkimus

TABLE OF CONTENTS

ABBREVIATIONS

ADC	Apparent diffusion coefficient
BAEP	Brainstem auditory evoked potential
CI	Confidence interval
CLD	Chronic lung disease
CONS	Coagulase negative Staphylococci
CP	Cerebral palsy
DNA	Deoxyribonucleic acid
DTI	Diffusion tensor imaging
e.g.	Exempli gratia
ELBW	Extremely low birth weight infants
FA	Fractional anisotrophy
FOV	Field of interest
IL	Interleukin
IVH	Intraventricular hemorrhage
LBW	Low birth weight
LPS	Lipopolysaccharide
MRI	Magnetic resonance imaging
NEC	Necrotising enterocolitis
OR	Odds ratio
PCR	Polymerase chain reaction
PLV	Periventricular leukomalacia
PPROM	Preterm premature rupture of the membranes
PROM	Premature rupture of the membranes
ROI	Region of interest
SD	Standard deviation
TE	Time echo
TNF	Tumour necrosis factor
TR	Time repetition
US	Ultrasound
VLBW	Very low birth weight
VLGA	Very low gestational age
WMD	White matter disease
WMI	White matter injury

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications referred to in the text by their Roman numerals (I-IV):

I Milla Reiman, MD, Harry Kujari, MD, Jonna Maunu, MD, Riitta Parkkola, MD, PhD, Hellevi Rikalainen, MD, Helena Lapinleimu, MD, PhD, Liisa Lehtonen, MD, PhD, Leena Haataja, MD, PhD, and the PIPARI Stydy Group Does Placental Inflammation Relate to Brain Lesions and Volume in Preterm Infants? J Pediatr 2008;152:642-7

II Milla Reiman, MD, Harry Kujari, MD, Eeva Ekholm, MD, Helena Lapinleimu, MD, PhD, Liisa Lehtonen, MD, PhD, Leena Haataja, MD, PhD, and the PIPARI Stydy Group Interleukin-6 Polymorphism is Associated with Chorioamnionitis and Neonatal Infections in Preterm Infants. J Pediatr 2008;153:19-24

III Milla Reiman, MD, Riitta Parkkola, MD, PhD, Helena Lapinleimu, MD, PhD, Liisa Lehtonen, MD, PhD, Leena Haataja, MD, PhD, and the PIPARI Study Group Interleukin-6 -174 and -572 genotypes and the volume of deep gray matter in preterm infants. Ped Res 2009;65:90-6

IV Milla Reiman, MD, Riitta Parkkola, MD, PhD, Reijo Johansson, MD, PhD, Satu K. Jääskeläinen, MD, PhD, Harry Kujari, MD, Liisa Lehtonen, MD, PhD, Leena Haataja, MD, PhD, Helena Lapinleimu, MD, PhD and the PIPARI Study Group Diffusion Tensor Imaging of the Inferior Colliculus and Brainstem Auditory-Evoked Potentials in Preterm Infants. Pediatr Radiol. 2009;39:804-9

1. INTRODUCTION

Chorioamnionitis is an important risk factor underlying preterm delivery (Romero et al., 2006; Goldenberg et al., 2000). Preterm infants, especially very low birth weight (VLBW) and very low gestational age (VLGA) infants, are at increased risk for brain lesions and neurological developmental problems. Placental inflammation has been suggested to be one of the factors leading to brain pathology in preterm infants (Kaukola et al., 2006; Polam et al., 2005; Vergani et al., 2000; Leviton et al., 1999; DiSalvo, 1998). Cytokines, on the other hand, are believed to be the link causing the deleterious effects of inflammation to the nervous system, as high levels of several pro inflammatory cytokines have been seen in cord blood (Tauscher et al., 2003), neonatal blood (Nelson et al., 2003; Heep et al., 2003), and cerebro spinal fluid (Ellisonn et al., 2005) in preterm infants subsequently developing brain lesions. The genetic regulation of inflammatory cytokines, i.e. the genetic composition of gene promoter regions, partly regulate the inflammatory response to stimuli, and has thus been suggested to also play a role in inflammation mediated brain injury. Indeed, single nucleotide polymorphisms in interleukin (IL)-6 promoter region (IL-6 -174 and -572 genotypes) have been connected with deviant neurological development in preterm infants (Harding et al., 2004; Resch et al., 2009; Harding et al., 2005).

The role of histological chorioamnionitis in the development of brain lesions in VLBW/VLGA infants is not sufficiently understood, and the relation of placental inflammation to brain volumes has not been investigated. The role of placental inflammation is also not known in relation to the functioning of the auditory pathway and hearing impairment in preterm infants. The significance of IL-6 promoter polymorphisms on inflammatory situations is complicated, and its relevance to the developing brain is not sufficiently investigated.

2. REVIEW OF THE LITERATURE

2.1. Very preterm delivery and infants

In Finland, 1 % of infants are born before 32 weeks of gestation (very low gestational age, VLGA) and 0.7 % are born below 1500 g (very low birth weight, VLBW) (National Birthregister, 2007). Prematurity is the leading cause of perinatal mortality. It has been estimated that prematurity accounts for 75 % of perinatal deaths and VLGA infants represent 60 % of these (Slattery et al., 2002). In Finland, 11.1 % of VLWB/VLGA infants die before they reach the age of 1 year (Rautava et al., 2007). Preterm birth causes long-term morbidities, the brain and lung being most vulnerable for the consequences of prematurity (Saigal et al., 2008). In the Finnish National Register Study including all VLBW/VLGA infants born between 2000 and 2003, 23.4 % of infants had at least one long-term morbidity during the first 3 to 6 years of life (Korvenranta et al., 2009). For example, obstructive airway disease was seen in 20 %, hearing loss in 2.5 %, and visual disorder in 3.8 % (Korvenranta et al., 2009). In the 1990s, approximately 25 % of survivors of very preterm birth had some neurological disabilities, the most immature survivors being most affected (Saigal et al., 2008). In the last decade, the neurological morbidity rate has decreased. In the subgroup of extremely low birth weight (ELBW, birth weight below 1000 g) infants, the prevalence of cerebral palsy (CP) has decreased from approximately 13 % to 5 % in the United States (Wilson-Costello et al., 2007), and in preterm infants born before 27 weeks of gestation in Finland from 19 % to 8.5 % (Korvenranta et al., 2009). In VLBW/VLGA infants born between 2000 and 2002 in Finland, the prevalence of CP has been 6.1 % (Korvenranta et al., 2009). A major cause of neurological disabilities in VLBW/VLGA infants is the high incidence of brain injury in this patient group. Intraventricular hemorrhage (IVH) is seen in approximately 26 % and periventricular leukomalasia (PVL) in 3 % of VLBW/VLGA infants (Vermont Oxford Network Database, 2008).

Preterm infants are also susceptible to inflammation and serious infections, such as chronic lung disease (CLD), necrotising enterocolitis (NEC), and septicaemia, largely because of immature compensatory anti-inflammatory response (Schultz et al., 2004) resulting in enhanced inflammatory response compared with term born infants(Yoon et al., 2003) and adults (Schultz et al., 2004). For example, in VLBW/VLGA infants, septicaemia is seen in 23 %, NEC in 7 %, and CLD in 34 % (Vermont Oxford Network Database, 2008). In Finnish preterm ELBW infants, septicaemia was seen in 22 %, NEC with bowel perforation in 9 %, and CLD in 39 % (Tommiska et al., 2001).

Preterm births are classified into two groups: spontaneous preterm births and labour induction or caesarean delivery for maternal or fetal indications (Goldenberg et al., 2008).

Indicated preterm labour accounts for approximately 30 % of preterm births (Goldenberg et al., 2008). Spontaneous preterm births are further classified into spontaneous preterm birth with intact membranes and preterm premature rupture of the membranes (PPROM), and they account for 45 % and 25 % of preterm births, respectively (Goldenberg et al., 2008; Slattery et al., 2002). Reasons for indicated preterm birth can be, for example, pre-eclampsia or eclampsia, and intrauterine growth restriction (Goldenberg et al., 2008; Slattery et al., 2002). Spontaneous preterm delivery can be caused by multiple factors, including infection or inflammation, vascular disease, and uterine overdistension, caused, for example, by multiple pregnancies (Goldenberg et al., 2008). Infection and inflammation become more frequent causes of preterm delivery as the gestational age decreases (Goldenberg et al., 2008). The causes of PPROM are often unknown, but a common risk factor is asymptomatic intrauterine infection (Andrews et al., 2000; Goldenberg et al., 2008; Goldenberg, 2002; Goldenberg et al., 2000). PPROM may also lead to ascending intrauterine infection, thus leading to preterm labour (Goldenberg et al., 2008).

2.2. Placental inflammation

2.2.1 Pathogenesis

Intrauterine infection is rare in term pregnancies but is commonly seen in preterm births (Anrews et al., 2000; Goldenberg et al., 2008; Goldenberg, 2002; Goldenberg et al., 2000). Placental infection is often asymptomatic, and therefore it is difficult to clinically diagnose before delivery. The diagnostic criteria of placental infection, i.e. clinical chorioamnionitis, varies between study groups, but the diagnosis is usually based on a combination of ruptured membranes, maternal fever, maternal tachycardia, fetal tachycardia, maternal leukocytosis, and foul-smelling amniotic fluid (Soper et al., 1996).

Histological chorioamnionitis, i.e. placental inflammation, on the other hand, is based on the appearance of neutrophiles in the placental tissue, and hence is not the direct equivalent of clinical chorioamionitis. The prevalence of histological chorioamnionitis is gestational age-specific and decreases almost linearly from 45-60 % in VLBW infants to 5-10 % in term pregnancies (Redline, 2004). Intrauterine inflammation within the uterus can be seen between the maternal tissue and the fetal membranes, within the fetal membranes, within the placenta, in the amniotic fluid, or within the umbilical cord (Goldenberg et al., 2000). Inflammation is seen as neutrophilic infiltration of the tissues, and is called chorioamnionitis when seen in fetal membranes, and funisitis when present in the umbilical cord (Goldenberg, 2002).

Microorganisms can invade the uterine cavity by ascending from the vagina and cervix, by migration from the abdominal cavity through the Fallopian tubes, hematogenously through the placenta, or by needle contamination at amniocentesis or other invasive procedures (Goldenberg et al., 2008; Romero et al., 2006). Ascending infection is the most important pathway, and vaginal organisms spread first to the choriodecidual space and then can cross the intact fetal membranes and spread to the amniotic fluid and finally infect the fetus (Goldenberg et al., 2008; Goldenberg et al., 2000). On the other hand, in the presence of premature rupture of the membranes (PROM), the amniotic epithelial surface is directly exposed to bacterial flora of the cervix, facilitating the spread of microorganisms directly into the uterine cavity (Redline, 2004). The bacteria causing placental inflammation with intact membranes are most often vaginal organisms of low virulence, including *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardrenella vaginalis*, Peptostreptococci and Bacteroides species (Goldenberg et al., 2008; Goldenberg, 2002; Goldenberg et al., 2000). After PROM, the organisms most frequently seen with chorioamnionitis are Group B Streptococci and *Escherchia coli* (Goldenberg, 2002; Goldenberg et al., 2000).

The timing of intrauterine infection is not sufficiently understood. During the fist trimester, the placenta and embryo are relatively well isolated from infection (Redline, 2004). At the turn of the fisrt and second trimester the organisms from the maternal circulation gain access to the fetally perfused placental villi with the onset of perfusion of the intervillous space (Redline, 2004). At 18 to 20 weeks of gestation, the gestational sac fuses with the uterine lining and organisms from the decidua vera gain access to the fetal membranes (Redline, 2004). Microbial colonization may precede conception, as is in the case of chronic endometritis, or micro-organisms may invade the uterus during the pregnancy (Goldenberg et al., 2008; Redline, 2004). After the fetal membranes seal the uterine cavity, the organisms usually no longer ascend from the vagina to the uterus (Goldenberg et al., 2000). It is thought that if the microorganisms are not destroyed by the mother's immune system in the few months after expanded membranes have sealed the uterine cavity, the infection becomes symptomatic and can lead to preterm labour or PROM (Goldenberg et al., 2000). However, in PROM, the uterine cavity is exposed to vaginal bacterial flora even later in the pregnancy.

2.2.2 Pathway to very preterm delivery

Approximately 25-40 % of all preterm births are complicated by intrauterine infection, while from spontaneous preterm deliveries occurring around 24 weeks of gestation, nearly all are associated with chorioamnionitis (Andrews et al., 2000; Goldenberg et al., 2008; Goldenberg, 2002; Goldenberg et al., 2002) Accordingly, intrauterine infection is an important mechanism leading to preterm delivery by activating the innate immune system (Goldenberg et al., 2000; Romero et al., 2006) In most cases

placental inflammation is asymptomatic, and PROM or preterm labour is the first sign of infection. Microbial invasion activates the decidua and fetal membranes to produce cytokines, which act with endotoxins and exotoxins to stimulate prostaglandin synthesis and release leading to uterine contractions (Goldenberg, 2002; Goldenberg et al., 2000; Romero et al., 2006). Cytokines also initiate neutrophil chemotaxis, infiltration, and activation, leading to synthesis and release of metalloproteases which then attack the fetal membranes and soften the cervix by remodelling the collagen fibers (Goldenberg, 2002; Goldenberg et al., 2000; Romero et al., 2006). The pathway leading from placental infection to preterm delivery is shown in Figure 1. The proinflammatory cytokines thought to be of major significance include tumour necrosis factor α (TNF- α), interleukin (IL)-1α, IL-1β, IL-6, IL-8, and granulocyte-colony stimulating factor (Goldenberg, 2002; Goldenberg et al., 2000; Romero et al., 2006). From anti-inflammatory cytokines, IL-10 is believed to have a role in maintaining pregnancy, and indeed, IL-10 expression has been shown to be reduced in association with chorioamnionitis (Romero et al., 2006). Other possible pathways may also include chorionic infection which decreases the activity of prostaglandin dehydrogenases, leading to larger amounts of prostaglandins reaching the myometrium and causing contractions (Goldenberg et al., 2000). On the other hand, in the case of fetal infection, the rise in fetal production of cortisol increases the production of prostaglandins and may thus lead to contractions (Goldenberg et al., 2000).

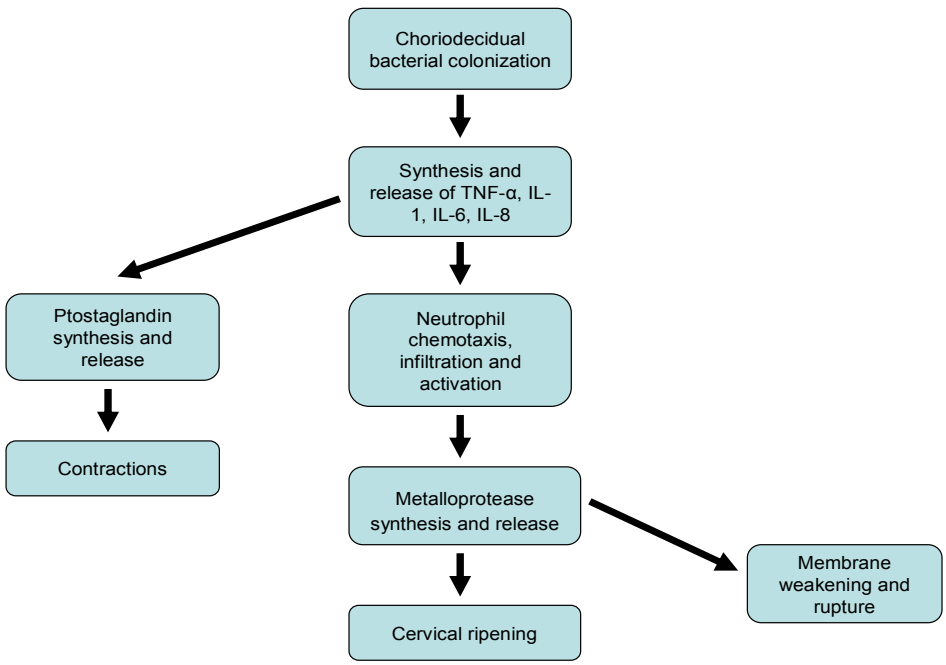

FIGURE 1. Potential pathway leading from placental infection to preterm delivery (Goldenberg, 2002).

2.2.3 Consequences to the central nervous system

Clinical and histological chorioamnionitis and maternal fever during delivery have all been associated with cerebral palsy (CP) in term born infants (Redline et al., 2000; Wu et al., 2003; Grether et al., 1997). Maternal fever and clinical chorioamnionitis have also been shown to be risk factors for birth asphyxia and neonatal encephalopathy including seizures, cerebral depression, and central nervous system dysfunction (Blume et al., 2008).

Among preterm infants the situation is less clear. Even though preterm infants are thought to be more susceptible to infections and inflammation, the reported consequences of intrauterine infections have been inconsistent in different studies. Several studies have shown an association between clinical (Alexander et al., 1998) and histological chorioamnionitis (DiSalvo, 1998; Kaukola et al., 2006; Leviton et al., 1999; Polam et al., 2005; Vergani et al., 2000) and brain pathology in VLBW/VLGA infants. Clinical chorioamnionitis has been connected with IVH and PVL in preterm VLBW infants (Alexander et al., 1998). Histological chorioamnionitis, on the other hand, has been associated with white matter damage (WMD) and IVH in VLBW/VLGA infants (Kaukola et al., 2006; Kaukola et al., 2005; Leviton et al., 1999; Polam et al., 2005; Vergani et al., 2000). In contrast, Kaukola et al. (Kaukola et al., 2006) found no association between histological chorioamnionitis and WMD in ELBW infants, and Holcroft et al. (Holcroft et al., 2003). were unable to associate clinical chorioamnionitis with IVH and PVL in VLBW infants.

Among more mature preterm infants the findings have been equally inconsistent. Clinical (Hatzidaki et al., 2009; Verma et al., 1997) and histological chorioamnionitis (Rocha et al., 2007) have been associated with brain pathology, but on the other hand, a few studies have been able to show only a trend (Kumazaki et al., 2002; Murata et al., 2005) or no association (Vergani et al., 2004) between histological chorioamnionitis and PVL or IVH. The association between clinical chorioamnionitis and PVL has also been contradicted (Spinillo et al., 1998).

The role of clinical and histological chorioamnionitis in preterm infants' neurological development has also been widely studied. There are studies showing an association between clinical and histological chorioamnionitis and CP in VLBW/VLGA infants (Constantine et al., 2007; Jacobsson et al., 2002) but several studies have found no association between clinical (Grether et al., 2003; O'Shea et al., 1998,) and histological chorioamnionitis (Gray et al., 2001; Grether et al., 2003) and in utero infection (Andrews et al., 2008) and CP. In a recent study by Andrews et al. (Andrews et al., 2008) with very preterm infants at the age of 6 years, in utero exposure to acute inflammation was not associated with CP, low IQ or neurodevelopmental delay, and they even reported a trend

indicating higher IQ in the presence on funisitis. PPROM was also associated with lower frequency of IQ <70 and major disability (Andrews et al., 2008).

It can be concluded from the literature that the findings about the neurological consequences of chorioamnionitis are contradictory. It is likely that the situation is more complex among immature preterm infants than in otherwise healthy term born infants, and that other, still unknown, factors play a role in determining the consequences of chorioamnionitis.

2.3. Cytokines

Cytokines are multifunctional pleiotrophic proteins that play a major role in initiating and mediating an inflammatory reaction. They also participate in cell-to-cell communication and cellular activation. Cytokines are released by many cells, including epithelial cells, endothelial cells, and the cells of the immune system. Cytokines are generally classified as being either pro-inflammatory or anti- inflammatory, and they have a wide spectrum of biological activities that help to coordinate the response to infection, including acting on the liver, bone marrow endothelia, hypothalamus, fat and muscle, and lymphocytes.(Janeway et al., 1997) In addition to being involved in immune responses, cytokines also have a functional role in the peripheral and central nervous system, as they modulate the action, differentiation and survival of neuronal cells, and on the other hand, participate in the pathogenesis of neurotoxic and neurodegenerative disorders (Szelényi et al., 2001).

2.3.1 Pathway to very preterm delivery

In association with intra uterine infection, the fetal membranes produce cytokines, which leads to uterine contractions, softening of the cervix, and membrane rupture (Goldenberg, 2002; Goldenberg et al., 2000; Romero et al., 2006). In addition to amniotic fluid (Menon et al., 2008) and cervical (Kurkinen-Räty et al., 2001) IL-6 concentrations associating with preterm birth, the association has also been reported between elevated levels of neonatal blood cytokines (L-1β, IL-6, soluble IL-6 receptor-α, IL-8, matrix metalloproteinase-9, transforming growth factor-β1) and preterm birth when compared to term born infants (Skogstrand et al., 2008). Accordingly, Skogstrand et al. (Skogstrand et al., 2008) suggested this to implie that inflammation of fetal origin might be a cause of preterm delivery, but it is more likely that it merely reflects the enhanced inflammatory response of preterm infants to delivery (Schultz et al., 2002; Yoon et al., 2003). Furthermore, elevation in maternal blood cytokines in mid pregnancy has been associated with preterm delivery before 35 weeks of gestation with or without histological chorioamnionitis (Gargano et al., 2008), and with delivery at 30-36 weeks of gestation (Curry et al., 2007).

2.3.2 Connection to placental inflammation and neonatal inflammatory diseases

Fetal cytokines seem to have a role in the placental inflammatory process, since elevated pro-inflammatory cytokine levels have been found in cord blood in the presence of chorioamnionitis (Dollner et al., 2002; Kashlan et al., 2000; Kaukola et al., 2006; Shalak et al., 2002; Tasci et al., 2006; Yanowitz et al., 2002; Yoon et al., 2000). High levels of interleukin IL-6, IL-8, IL-1β, and tumour necrosis factor (TNF)α in full term infants (Dollner et al., 2002; Tasci et al., 2006) and elevated IL-6 concentrations in preterm infants (Pacora et al., 2002; Yoon et al., 2000) as well as elevated IL-6 and IL-8 concentrations in a subgroup of VLBW/VLGA infants (Kashlan et al., 2000; Kaukola et al., 2006; Yanowitz et al., 2002) have all been associated with histological chorioamnionitis and/or funisitis. High levels of blood cytokines during the perinatal period have also been associated with histological chorioamnionitis in very preterm infants (Paananen et al., 2008). In addition, high cord blood levels of IL-6 in term born infants have been connected with clinical chorioamnionitis (Shalak et al., 2002). Elevated IL-6 levels have also been found in the cerebrospinal fluid of infants with histological and clinical chorioamnionitis (Laborada et al., 2005). Since proinflammatory cytokines, at least in healthy term placentas, are not transmitted through the placenta (Aaltonen et al., 2005), these findings suggest that chorioamnionitis can also lead to fetal inflammatory response. Although, it has to be kept in mind that cytokine levels are highly affected by several external factors, delivery being one of them (Kilpinen et al., 2001), making the studies based on cytokine levels prone to errors.

In addition to chorioamnionitis, fetal cytokines have been studied in relation to neonatal inflammatory diseases. Elevated cord blood IL-6 concentrations have been measured in preterm infants subsequently developing sepsis, congenital pneumonia or necrotizing enterocolitis (NEC) (Weeks et al., 1997) and in VLGA infants with increased risk for systemic inflammatory response syndrome and NEC (Goepfert et al., 2004). Elevated amniotic fluid TNFα has also been associated with necrotising enterocolitis (NEC) in infants born before 34 weeks of gestation (Hitti et al., 2001). In addition, elevated cord blood IL-6 and IL-8 levels (An et al., 2004) and elevated blood IL-8 in the perinatal period (Paananen et al., 2008) have been associated with progression of chronic lung disease (CLD) in VLBW/VLGA infants.

2.3.3 Consequences to the central nervous system

Cytokines are known to modulate the action, differentiation and survival of neuronal cells, and on the other hand, cytokine overexpression has been reported in the pathogenesis of neurotoxic and neurodegenerative disorders (Szelényi et al., 2001). High levels of IL-1β, IL-6, and IL-8 in cord blood have been associated with development of intracerebral hemorrhage in VLGA infants (Tauscher et al.,

2003), and high concentrations of proinflammatory cytokines in neonatal blood have been seen in VLGA infants with later brain ultrasound abnormalities (Heep et al., 2003; Nelson et al., 2003). In addition, elevated IL-6, IL-1β, and TNFα levels in amniotic fluid have been seen in pretem infants born before 35 weeks of gestation subsequently developing white matter lesions (Yoon et al., 1997) and grade 3-4 intraventricular hemorrhage (Hitti et al., 2001). On the other hand, Ellison et al. (Ellisonn et al., 2005) showed that in VLGA infants, cytokine concentrations in blood did not correlate to concentrations in cerebrospinal fluid (CSF), suggesting that plasma cytokine levels may poorly reflect the cerebral inflammatory response in preterm infant. They found an association between high CSF levels of IL-6, IL-10, and TNFα and white matter injuries (WMI), but no correlation between neonatal blood cytokines and WMI (Ellisonn et al., 2005).

Since neonatal cytokines have been associated with brain injury in preterm infants, the role of cytokines in neurological development has also been under intensive research. In full term infants, higher neonatal blood levels of IL-1β, IL-6 , IL-8, and TNFα have been associated with abnormal neurodevelopmental outcome at 30 months of age (Bartha et al., 2004). In addition, elevated IL-6 levels in neonatal blood at 6 hours of age have been reported to associate with lower scores in modified Dubowitz neurological examination and with hypoxic ischemic encephalopathy and/or seizures (Shalak et al., 2002). In contrast, in studies with preterm infants, neonatal cytokines have not consistently been associated with abnormal neurodevelopmental outcome. Kaukola et al. (Kaukola et al., 2006) found no association between cord blood IL-1α, IL-1β, IL-6, IL-8, and TNFα levels and neurodevelopmental outcome in ELBW infants at two years of corrected age. Nelson et al. (Nelson et al., 2003) evaluated the association between neonatal cytokines, including IL-1, IL-6, IL-8, and TNFα, and later diagnosis of CP in VLBW infants. They found that none of the measured cytokine concentrations were related to CP. However, in a recent study, high levels of IL-6 and TNF-α in cord blood have been associated with low scores in the Bayley Scales of Infant Development psychomotor developmental index and TNF-α with CP in very preterm infants at the corrected age of 2 years (Hansen-Pupp et al., 2008).

2.3.4 Interleukin-6

IL-6 is expressed in multiple cell types, and the gene has a complex transcriptional regulation. Table 1 shows the wide variety of actions of IL-6.

TABLE 1. Actions of IL-6 (Papanicolaou et al., 1998)

Hematologic	Proliferation of multipotential hematopoietic progenitors
	Myeloma and plasmacytoma cell growth
Immunologic	Differentiations and maturation of B cells (B-cell stimulating factor 2)
	Production of immunoglobulin by B cells
	Proliferation and differentiation of T cells
Hepatic	Hepatocyte stimulation
	Introduction of various genes of the acute-phase response (C-reactive protein, haptoglobin, fibrinogen)
Neurologic	Nerve cell differentiation
	Gliosis (in transgenic mice)
Cardiac	Myocardial hypertophy
Endocrine	Induction of thermogenesis (endogenous pyrogen)
	Stimulation of the hypothalamic pituitary-adrenal axis
	Stimulation of vasopressin secretion
	Stimulation of growth hormone secretion
	Suppression of the thyroid axis
	Suppression of serum lipid levels
	Osteoporosis (postmenopausal or due to hypogonadism)

IL-6 has been shown to be essential for normal brain function, for example memory processes (Braida et al., 2004), and protection from brain injury (Loddick et al., 1998; Matsuda et al., 1996; Penkowa et al., 2000; Winter et al., 2004). IL-6 has been suggested to act as a neuroprotective agent in response to head trauma in adult patients (Winter et al., 2004), and in animal models it has been shown to be an important endogenous inhibitor of neuronal death during cerebral ischemia (Loddick et al., 1998) and act as a trophic agent on ischemic hippocampal neurons (Matsuda et al., 1996). IL-6 –type cytokines also act as factors influencing the development of neurons and glial cells in the central nervous system, and have been shown to be essential at least for the development of astrocytes (Koch et al., 2007; Nakanishi et al., 2007; Taga et al., 2005). On the other hand, long-term overexposure to IL-6 has been suggested to be harmful for central nervous system, and can interfere with the neurogenesis of adult transgenic mice (Vallières et al., 2002) and cause neuronal damage, reactive astrocytosis, and proliferative angiopathy leading to profound neurologic disease (Campbell et al., 1993).

2.3.4.1 IL-6 promoter polymorphism and inflammatory response

The production of IL-6 is controlled at the translational level, and genetic factors partly regulate the level of IL-6 response. Single nucleotide polymorphisms in the IL-6 gene promoter region at positions -174 (G/C) and -572 (G/C) have been shown to affect IL-6 expression. IL-6 G at position -174 has been found to associate with elevated IL-6 expression in HeLa cells both unstimulated and after stimulation with IL-1 and lipopolysaccharide (LPS) (Fishman et al., 1998). Leukocytes stimulated with endotoxins

(Rivera-Chavez et al., 2003) and macrophages both unstimulated and after stimulation with leishmania antigen (Castellucci et al., 2006) have also shown elevated IL-6 response in association with the GG genotype. In addition, in vivo studies have shown that the IL-6 -174 GG genotype is associated with higher levels of IL-6 in patients with sepsis/septic shock (Tischendorf et al., 2007), pancreatic adenocarcinoma/chronic pancreatitis (Talar-Wojnarowska et al., 2008), non-functioning pancreatic endocrine tumors/gastrointestinal neuroendocrine tumors (Berković et al., 2007), and in generally healthy adults (Raunio et al., 2007). On the other hand, in patients with sepsis/septic shock, CC genotype has associated with higher IL-6 response ex vivo after stimulation with LPS in a whole blood assay (Tischendorf et al., 2007). In vivo, CC genotype has been connected with elevated production of IL-6 in patients with atrial fibrillation (Marcus et al., 2008), and in neonates and neonatal monocytes stimulated with lipopolysaccharide (Kilpinen et al., 2001). In addition, C allele has been shown to associate with a higher IL-6 level in patients with abdominal aortic aneurysms (Jones et al., 2001) and cardiovascular disease as well as in their healthy control group (Jenny et al., 2002). Considering the discrepancy of the findings described above, it seems that the regulation of IL-6 synthesis is a complex process, and many factors need to be taken into account. Some studies have even reported no association between IL-6-174 polymorphism and IL-6 response in vivo (Potaczek et al., 2007; Taudorf et al., 2008) and ex vivo (Heesen et al., 2002; Kiszel et al., 2007), and different responses in control versus patient groups (Potaczek et al., 2007) and in neonates versus adults (Kilpinen et al., 2001). It has been suggested that genetic polymorphism in the promoter area of IL-6 gene influences IL-6 transcription not by a simple additive mechanism but rather through complex interactions determined by the haplotype (Terry et al., 2000).

C allele at the position -572 has been associated with increased IL-6 synthesis in the Western population after coronary artery by-pass surgery (Brull et al., 2001) and in patients with acute coronary syndrome (Mälarstig et al., 2007). In addition, in the Chinese Han population, C allele was seen with higher plasma IL-6 levels when stimulated with LPS (Gu et al., 2008). On the other hand, in the Korean population, it has been shown that the GG genotype associates with higher levels of IL-6 in healthy subjects (Koh et al., 2009; Shin et al., 2007) and in men with coronary artery disease not taking a lipid-lowering drug (Jang et al., 2008), suggesting a possible racial difference in the regulation of IL-6 synthesis.

2.3.4.2 IL-6 promoter polymorphism in association with infections

IL-6-174 polymorphism has been associated with the incidence, clinical course, and outcome of several infectious diseases and autoimmune disorders. Sepsis, a consequence of inadequate immune system activation, has been associated with IL-6-174 GG genotype in preterm infants (Ahrens et al., 2004; Harding et al., 2003). GG genotype has also been

associated with the common cold (Nieters et al., 2001) and recurrent otitis media in children (Emonts et al., 2007; Patel et al., 2006). A trend between IL-174 G and histologic chorioamnionitis has also been found (Speer et al., 2006) However, the findings of the role of IL-6-174 polymorphism on infections have not been consistent. The C allele has also been associated with increased incidence of sepsis in pediatric intensive care patients (Michalek et al., 2007), with increased incidence of late blood stream infections in African American mechanically ventilated VLBW infants (Baier et al., 2006), and with septic shock in adults (Tischendorf et al., 2007). In addition, Göpel et al. (Göpel et al., 2006) and Schlüter et al. (Schlüter et al., 2002) found no association between the incidence of sepsis and IL-6-174 genotype in VLBW infants. The association between maternal IL-6 -174 genotypes and the incidence of preterm delivery has been studied showing no association between IL-6 -174 genotypes and preterm birth (Annells et al., 2004; Lamie et al., 2005; Mattar et al., 2006; Moura et al., 2009; Stonek et al., 2008).

The association of IL-6-572 genotypes with infectious diseases has been less explored. In two available studies, IL-6-572 C associated with higher incidence of sepsis in pediatric intensive care unit patients (Michalek et al., 2007) and adult trauma patients (Gu et al., 2008).

2.3.4.3 IL-6 promoter polymorphism and brain lesions and neurological development

Since IL-6 has a variety of protective as well as deleterious effects on the central nervous system, it is plausible that IL-6 promoter polymorphism, affecting the synthesis of IL-6, would play a role in both brain development and pathology. In Caucasians, IL-6-174 G and -572 C have both been connected with intracranial aneurysmal disease (Morgan et al., 2006). In the Chinese population, on the other hand, IL-6-572 GG genotype has been associated with intracranial aneurysms (Sun et al., 2008), possibly indicating a racial difference in the regulation of IL-6 synthesis. In studies concerning preterm infants, IL-6-174 and -572 polymorphisms have been connected with brain pathology and neurological development. Harding et al. (Harding et al., 2004) found an association between IL-6-174 CC genotype and brain pathology and disability at 2 and 5,5 years in very preterm infants. Göpel et al. (Göpel et al., 2006), on the other hand, found no association between IL-6-174 polymorphisms and brain pathology in VLBW infants. IL-6 -174 CC and GC genotypes have also been associated with mental retardation in preterm infants with cystic PVL, suggesting the IL-6 -174 polymorphism to modify the severity of perinatal brain injury (Resch et al., 2009). IL-6-572 C has not been shown to associate with brain pathology in very preterm infants, but has been connected with impaired cognitive development at the age of 2 and 5,5 years (Harding et al., 2005).

The literature related to the associations of IL-6 -174 and -572 genotypes with brain lesions and neurological development has been summarised in Table 2.

TABLE 2. The literature related to the associations of IL-6 -174 and -572 genotypes with brain lesions and neurological development.

IL-6 -174	**-174 G**	**-174 C**
Harding et al., 2004 (N=148)		IL-6-174 CC associated with brain pathology and disability at 2 and 5,5 years in VLGA infants
Göpel et al., 2006 (N=1206)	No association between IL-6-174 polymorphisms and brain pathology in VLBW infants	
Resch et al., 2009 (N=56 cases + 46 preterm and 395 term controls)		IL-6 -174 CC and GC associated with mental retardation in preterm infants with cystic PVL
Morgan et al., 2006 (N=91 cases + 2720 controls)	IL-6 -174 G associated with intracranial aneurysmal disease in Caucasians	
IL-6 -572	**-572 G**	**-572 C**
Harding et al., 2005 (N=113)		IL-6 -572 C associated with impaired cognitive development at 2 and 5,5 years in VLGA infants
Sun et al., 2008 (N=240 cases + 240 controls)	IL-6-572 GG associated with intracranial aneurysms in the Chinese	
Morgan et al., 2006 (N=91 cases + 2720 controls)		IL-6 -572 C associated with intracranial aneurysmal disease in Caucasians

2.4. Brain pathology in preterm infants

2.4.1 Brain injury

Preterm infants are at an increased risk for brain injury. Whether preterm birth and brain damage are both consequences of previous events, or whether preterm birth is an independent cause of brain damage, is not known (Arpino et al., 2005). Brain damage in preterm infants primarily affects the white matter (Inder et al., 2000; Rees et al., 2005), and the most common type of damage is PVL, IVH, and periventricular hemorrhagic infarction (Arpino et al., 2005; Whitelaw et al., 2001). In recent years, the incidence of cystic PVL visible in cranial ultrasound (US) examination has been 3 % and IVH 26 % in VLBW infants (Vermont Oxford Network Database, 2008). Diffuse, non-cystic PVL has been estimated to be more common than the cystic type in VLBW infants (Rees et al., 2005; Volpe, 2003).

PVL is necrosis of white matter in the typical location in the posterior periventricular white matter adjacent to the lateral ventricle and in the white matter adjacent to the foramina of Monro (Barkovich, 1995). PVL can be caused by focal periventricular necrosis or more

diffuse cerebral white matter injury (Inder et al., 2000). The focal form usually occurs in the area of the end zones of the long penetrating arteries adjacent to the lateral ventricles, and undergoes cavity formation over 1-3 weeks (Inder et al., 2000; Rees et al., 2005) Diffuse white matter damage less commonly forms cysts, and may remain undetected by cranial US examination (Inder et al., 2000). Magnetic resonance imaging (MRI) studies have shown diffuse damage to be very common in premature infants (Rees et al., 2005; Volpe, 2003). PVL has also been suggested to be related to a disturbance in subsequent cortical neuronal development (Inder et al., 2000) and deep gray matter injury (Rees et al., 2005), and the focal form can selectively damage the periventricular crossroads of growing thalamocortical and associative pathways (Kostović et al., 2006). Diffuse white matter injury, on the other hand, predominantly affects developing oligodendrocytes and the subplate zone (Kostović et al., 2006).

IVH in preterm infants usually originates from the subependymal germinal matrix, and often ruptures through the ependyma into the ventricles (Whitelaw et al., 2001). IVH is divided into three grades, grade 1 confined to the subependymal matrix, grade 2 in the ventricle without distending it, and grade 3 extending more than half the length of the lateral ventricle distending the ventricle (Whitelaw et al., 2001). IVH may also be accompanied by periventricular hemorrhagic infarction, which is seen in 15-20 % of infants with IVH (Inder et al., 2000; Whitelaw et al., 2001). Periventricular hemorrhagic infarction is hemorrhagic necrosis of the periventricular white matter from the venous origin (Inder et al., 2005). It is usually asymmetric, and follows the distribution of the medullary veins in periventricular white matter, and in 80 % of cases is associated with IVH (Inder et al., 2000; Whitelaw et al., 2001). In fact, it has been suggested that IVH/germinal matrix hemorrhage leads to obstruction of terminal veins, leading to hemorrhagic venous infarction and thus to periventricular hemorrhagic infarction (Inder et al., 2000).

The two pathways commonly believed to cause brain injuries in preterm infants are prenatal hypoxic/ischaemic injury and inflammatory/infective insults (Arpino et al., 2005; Inder et al., 2000; du Plessis et al., 2002; Rees et al., 2005). Preterm infants are particularly vulnerable for brain injury, mainly because of the immaturity of the central nervous system. The vessels penetrating the cerebral wall are highly undeveloped at 24 to 28 weeks of gestation, forming vascular end zones and border zones in cerebral white matter, and this exposes preterm infants born at this stage to injury in the presence of cerebral ischemia (Inder et al., 2000). In addition, preterm infants have impaired cerebrovascular regulation, and at least in clinically unstable infants, the rate of cerebral blood flow is lowered during systemic hypotension (Inder et al., 2000). Immature oligodendrocytes have also been suggested to be vulnerable in the premature brain, for example to cerebral ischemia, glutamate, inflammatory cytokines, and free radical attack, whereas the mature oligodendrocytes seem to be more resistant (Inder et al., 2000; du Plessis et al., 2002; Rees et al., 2005). Cytokine toxicity is most marked in

the most immature oligodendrocytes, and can be mediated through direct cytotoxicity, inhibiting the differentiation of oligodendrocyte precursors, stimulating oligodendrocyte apoptosis, and causing vacuolar myelin degeneration (du Plessis et al., 2002). It has also been suggested that cytokine toxicity may be caused by disturbances in glutamate transport (du Plessis et al., 2002). In association with IVH, the factors making premature brain particularly vulnerable include the fragility of the germinal matrix vascular bed (Whitelaw et al., 2001). During the third trimester, the germinal matrix produces glial precursors of oligodendroglia and astrocytes, and requires a rich blood supply for rapid cell division (Whitelaw et al., 2001). The vessels do not have extensive connective tissue support, and are thus prone to blood pressure changes and hypoxic-ischemic reperfusion and free radical injury (Whitelaw et al., 2001).

2.4.2 Brain volumes

Several studies have shown that preterm infants have smaller regional brain volumes at term age compared with term born infants both in the white matter and gray matter regions. For example, reductions in cerebral cortical gray matter and deep gray matter have been reported in preterm infants at term age (Boardman et al., 2006; Inder et al., 2005; Srinivasan et al., 2007). More precisely, the parieto-occipital, inferior occipital, sensorimotor, orbitofrontal, and premotor regions have been shown to be smaller in preterm infants compared with term born infants (Peterson et al., 2003; Thompson et al., 2007), as have thalamic and lentiform volumes (Inder et al., 2005; Srinivasan et al., 2007). Smaller cerebellar volumes have also been reported in preterm infants (Limperopoulos et al., 2005; Shah et al., 2006). In addition to studies at term age, the brain volumes of preterm infants have also been investigated in childhood (Peterson et al., 2000; Reiss et al., 2004), adolescence (Allin et al., 2001; Isaacs et al., 2000; Kesler et al., 2008; Nosarti et al., 2008; Nosarti et al., 2002) and adulthood (Fearon et al., 2004). The association between prematurity and reduction in volumes of cortical gray matter (Kesler et al., 2008; Nosarti et al., 2008; Nosarti et al., 2002; Peterson et al., 2000; Reiss et al., 2004), deep gray matter (Isaacs et al., 2000; Kesler et al., 2008; Nosarti et al., 2008; Nosarti et al., 2002; Peterson et al., 2000), white matter (Fearon et al., 2004; Kesler et al., 2008; Nosarti et al., 2008; Peterson et al., 2000; Reiss et al., 2004), and the cerebellum (Allin et al., 2001; Nosarti et al., 2008; Peterson et al., 2000) was seen in all of the studies. However, the pathogenesis and etiology of brain volume reductions in preterm infants is not well known. Suggested etiologies have been low gestational age (Boardman et al., 2006; Inder et al., 2005; Limperopoulos et al., 2005; Nosarti et al., 2008), prolonged supplemental oxygen dependence (Boardman et al., 2007; Thompson et al., 2007), treatments used in neonatal intensive care (i.e. postnatal steroids and indometacine treatment) (Thomson et al., 2008), and brain pathology (Boardman et al., 2006; Inder et al., 2005; Inder et al., 1999; Limeropoulos et al., 2005; Lin et al., 2001; Srinivasan et al., 2007; Thompson et al., 2008; Thompson et al., 2007; Vasileiadis et al.,

2004). For example, moderate or severe WMI has been associated with reduced cortical gray matter volume (Inder et al., 2005), hippocampal volume (Thompson et al., 2008), and the volumes of the dorsal prefrontal, sensorimotor, midtemporal, parieto-occipital, and inferior occipital regions (Thompson et al., 2007). Boardman et al. (Boardman et al., 2006) and Srinivasan et al. (Srinivasan et al., 2007) have both shown diffuse WMI to associate with smaller volumes of thalami and lentiform nuclei, and PVL has been shown to associate with smaller volumes in the thalami, cortical gray matter, and myelinated white matter (Inder et al., 1999; Lin et al., 2001). Cerebellar volumes have been shown to be reduced in association with brain pathology, even in the absence of cerebellar injury (Limperopoulos et al., 2005), and even uncomplicated IVH (grades I-II) has been suggested to reduce the volumes of cortical gray matter (Vasileiadis et al., 2004). Recent reports have suggested that it is possible that instead of prematurity itself, the pathologies associated with prematurity are responsible for reductions in brain volumes at term age. Boardman et al. (Boardman et al., 2007) showed that in preterm infants born before 33 weeks of gestation without focal parenchymal lesions or posthemorrhagic ventricular dilatation, the cerebral volume was not reduced at term age compared with term born infants. Hippocampal volumes have also been seen to be preserved in preterm infants without WMI, indometacine treatment, or postnatal steroids (Thompson et al., 2008), and cortical gray matter volumes have been shown to be normal in preterm infants with no brain lesions (Mewes et al., 2006). On the other hand, Kesler et al. (Kesler et al., 2008) have shown reduced white and gray matter in 12 year old preterm boys with normal neonatal US findings. In the light of recent publications, it is impossible to know whether prematurity is the cause of reduced brain volumes in preterm infants later in their lives, or whether the cause to reduced volumes is brain injuries and other diseases or treatments associated with prematurity, as the majority of the studies have been carried out without a healthy preterm control group.

The reductions in regional brain volumes have been shown to affect the development of preterm infants. Cognitive development at 18 to 20 months corrected age has been shown to be delayed in preterm infants with smaller sensorimotor and midtemporal white matter volumes (Peterson et al., 2003). A decrease in cerebral gray matter (Nosarti et al., 2008; Reiss et al., 2004), cortical (Peterson et al., 2000), and cerebellar volume (Allin et al., 2001) has been associated with poor cognitive performance at 8 and 14 to 15 years of age. Memory functions in 2 (Beauchamp et al., 2008) and 13 (Isaacs et al., 2000) year old preterm infants have been shown to be affected by reduction of hippocampal volumes. In addition, the reduction of caudate nuclei (Abernethy et al., 2004; Abernethy et al., 2002; Nosarti et al., 2005) and thalamus volume (Gimenez et al., 2006) has been suggested to cause developmental and behavioural problems in preterm infants.

2.4.3 Brain maturation

The level of brain maturation in preterm infants, and other subtle changes in the brain structure, is difficult, and in some cases impossible, to measure using conventional MRI or US. Diffusion tensor imaging (DTI), which is based on water diffusivity, is a fairly novel method to examine subtle changes in brain white matter structure. The apparent diffusion coefficient (ADC) value in DTI reflects free movement of water in a tissue, whereas the fractional anisotropy (FA) value reflects the directionality of diffusion (Beaulieu et al., 2002). Anisotropy in neural tissue is due to dense packing of axons, axonal membranes and myelin sheaths and can thus be used in estimating white matter maturation and myelination (Beaulieu et al., 2002). Brain water content decreases with increasing gestational age, and white matter complexity increases with increasing myelination, constricting water motion (Dudink et al., 2008). Indeed, ADC values of several brain white matter areas have been shown to decrease (Hüppi et al., 1998; Neil et al., 1998) and FA values increase (Dudink et al., 2007; Gilmore et al., 2004; Hüppi et al., 1998; Neil et al., 1998) with increasing gestational age. Brain maturation occurring after the perinatal period can also be detected with DTI. Studies of white matter tracts have shown that the development of decreasing ADC and increasing FA with increasing age can be seen from preterm infants born at 28 gestational weeks into childhood, adolescence, and young adulthood (Berman et al., 2005; Forbes et al., 2002; McGraw et al., 2002; Schmithorst et al., 2002; Schneider et al., 2004; Snook et al., 2005; Zhang et al., 2005). In addition to providing information of normal brain maturation, DTI is also feasible in studying brain injury, and even subtle changes in white matter structure. Preterm infants at term age, compared with term born infants, have been shown to have higher ADC values (Hüppi et al., 1998) and lower FA values (Anjari et al., 2007; Hüppi et al., 1998) in several white matter regions, suggesting that preterm birth disrupts normal brain development. Lower FA values of a wide range of white matter structures have even been seen in VLBW infants in childhood (Yung et al., 2007) and adolescence (Skranes et al., 2007; Vangberg et al., 2006). In addition, WMI has been shown to disturb white matter maturation, causing changes in ADC and FA values. For example, ADC has been shown to be higher (Counsell et al., 2003) and FA lower (Hüppi et al., 2001) in infants with WMI compared with controls. In addition, in newborns with WMI, ADC has been shown to increase or fail to decline, and FA has failed to increase with increasing age (Miller et al., 2002). Hüppi et al. (Hüppi et al., 2001) also showed that focal white matter injury can have major deleterious effects on the development of fibre tracts, resulting in low FA values in areas containing descending fibres from the injured white matter.

2.5. Hearing impairment in preterm infants

Prematurity (D'Mello et al., 1995; Korres et al., 2005; Mayr et al., 1994; Singh et al., 1998) and low birth weight (D'Mello et al., 1995; Korres et al., 2005; Morlet et al.,

1998; Van Naarden et al., 1999; Vohr et al., 2000) have been related to an increased risk for sensorineural hearing impairment, and up to 27 % of preterm VLBW infants have been found to have peripheral and/or central hearing impairment at term age (Jiang et al., 2001) Common co-morbidities associated with prematurity, including brain lesions, infections, and inflammatory diseases, have also been associated with greater risk for hearing impairment. Hearing deficits have been associated with congenital infections (Das et al., 1996; Yoshikawa et al., 2004), especially congenital cytomegalovirus infections (2006, Barbi et al., 2006; Barbi et al., 2003; Fowler et al., 2006; Iwasaki et al., 2007; Ogawas et al., 2007; Wen et al., 2002). Meningitis (Bao et al., 1998; Das et al., 1996; Meyer et al., 1999; Singh et al., 1998), sepsis (Marlow et al., 2000; Meyer et al., 1999; Singh et al., 1998; Stoll et al., 2004), and necrotising enterocolitis (NEC) (Stoll et al., 2004) have all been shown to be risk factors for sensorineural hearing impairment. The role of chorioamnionitis on hearing impairments, on the other hand, is not well established. One study group searched for an association between chorioamnionitis and hearing impairment (Fung et al., 2003), and found that chorioamnionitis did not associate with the incidence of hearing impairment in extremely premature infants.

Brain lesions have been associated with hearing impairment in preterm infants (Marlow et al., 2000; Patra et al., 2006), and even uncomplicated IVH has been associated with deafness (Patra et al., 2006). In addition, preterm infants with hearing loss had smaller volume of brain stem at term age compared with infants with no neurosensory disability (Valkama et al., 2001). Myelination delays and migrational abnormalities of the central nervous system have also been seen in children with sensorineural hearing loss (Lapointe et al., 2006). DTI has also been used in imaging subjects with hearing impairment. Three groups have reported reduced FA of the inferior colliculus in patients with sensorineural hearing impairment compared with normally hearing controls (Chang et al., 2004; Lin et al., 2008; Wu et al., 2009), suggesting the inferior colliculus to be the most sensitive area to neuronal damage in the auditory pathway. DTI has not been used in the investigation of hearing impairment of preterm infants.

2.5. Gaps in the current literature

Placental inflammation is an important risk factor for preterm delivery and it has also been suggested to associate with brain pathology and neurological development in preterm infants. However, the relationship between placental inflammation and brain development is inadequately understood, and the association of chorioamnionitis on regional brain volumes has not been investigated. The role of placental inflammation is also not known in relation with the development and functioning of the auditory pathway in preterm infants. Cytokines have been suggested as a possible link causing the deleterious effects of inflammation to the nervous system but the role of their genetic regulation is not sufficiently known in preterm infants.

3. AIMS OF THE STUDY

The aims of this study were:

1. To evaluate the association between histological inflammation of the placenta and brain findings in ultrasound examinations and regional brain volumes in MRI in VLBW/VLGA infants.

2. To evaluate whether genotypes of interleukin-6 (IL-6) gene promoter positions -174 and -572 associate with histological chorioamnionitis and neonatal inflammatory diseases in VLBW/VLGA infants.

3. To test the novel hypothesis of a possible association between IL-6 -174 and -572 genotypes and brain volumes and to evaluate their role on brain findings in VLBW/VLGA infants.

4. To investigate whether placental inflammation and neonatal inflammatory diseases affect the auditory pathway.

4. PATIENTS AND METHODS

This study is a part of the larger multidisciplinary PIPARI project (Development and Functioning of Very Low Birth Weight Infants from Infancy to School Age), where a 6-year cohort of VLBW/VLGA infants (n=274, 42 deceased) are being followed until school age in Turku University Central Hospital, Finland.

4.1. Patients

Inclusion criteria for the main study were 1) infant's birth weight ≤1500 grams (VLBW), and after April 2004, infant's birth weight ≤1500 grams or gestational age below 32 weeks (VLGA), 2) infant's parents spoke and understood written Finnish or Swedish, and 3) families lived inside the hospital catchment area.

For study I, the study population consisted of 121 infants born between January 2002 and March 2006. The exclusion criteria were 1) the placental sample was not available and /or 2) brain imaging was not available in surviving infants. Seventy two infants were excluded due to missing placenta (n=71) or brain imaging studies (n=1).

For study II, the study population consisted of 107 infants born between January 2002 and October 2006. The exclusion criteria were: 1) no placental sample was available and /or 2) no DNA sample was available. One hundred and one infants were excluded owing to an unavailable placenta (n=41) or DNA sample (n=31) or both (n=29). In multifetal pregnancies, the second and/or third infant was excluded from the analyses if the infants had the same gender, the same blood type and both IL-6 genotypes were similar. Altogether, 10 infants were excluded for this reason.

For study III, the study population consisted of 175 infants born between January 2001 and December 2006. The exclusion criteria were 1) DNA sample was not available and/or 2) brain imaging was not available. Ninety infants were excluded due to a missing DNA sample (n=89) or brain imaging (n=1). In multifetal pregnancies the second and/or third child were excluded from the analyses if the infants had the same gender, had the same blood type and both IL-6 genotypes were similar. Altogether, 9 infants were excluded for these reasons.

For study IV, the study population consisted of 135 infants born between January 2002 and December 2006. In this study we included infants who had had at least 2 of the 3 following examinations performed: 1) histological examination of the placenta, 2) DTI of the inferior colliculus, 3) BAEP recording. Altogether 53 infants of the eligible infants were excluded because of missing examinations (no placental samples and MRI for DTI (n=36), no placental samples and BAEP measurements (n=1), no MRI for DTI and BAEP measurements (n=11), all examinations missing (n=5)).

Parental consent was obtained after verbal and written information. The present study was approved by the Ethics Committee of the Hospital District of the South-West Finland in June 2001.

4.2. Methods

4.2.1 Data collection

The clinical data were collected prospectively as a part of the PIPARI protocol (see Table 3). Gestational age was estimated according to first or second trimester ultrasonography, which is performed routinely in Finland. The mother was defined as having clinical chorioamnionitis if her C-reactive protein value was pathological, she had fever, or premature rupture of membranes (PROM) over 18 hours prior to the delivery. In study I, if PROM was the only sign of clinical infection without histological chorioamnionitis, the mother was excluded from the clinical chorioamnionitis group. Neonatal inflammatory diseases, including septicaemia, chronic lung disease (CLD), and necrotising enterocolitis (NEC) were defined according to the Vermont-Oxford Network definitions as given for each year (Studies II and IV).(Vermont Oxford Network Database, 2005)

TABLE 3. Characteristics of the study infants. Means (SD) and [min, max] are presented for gestational age, birth weight and birth weight z score.

Characteristics	Study I n=121	Study II n=107	Study III n=175	Study IV n=135
Male	69 (57 %)	65 (61 %)	102 (58 %)	84 (62 %)
Singleton	90 (74 %)	79 (74 %)	124 (71 %)	93 (69 %)
Twin	25 (21 %)	23 (21 %)	43 (25 %)	36 (27 %)
Triplet	6 (5 %)	5 (5 %)	8 (5 %)	6 (4 %)
Caesarean section	76 (63 %)	69 (64 %)	107 (61 %)	80 (59 %)
Gestational age (weeks)	28+4 (2+6) [22+5, 36+1]	29+0 (2+5) [23+5, 36+1]	29+0 (2+5) [23+0, 36+1]	29+1 (2+5) [23+3, 36+1]
Birth weight (g)	1047 (306) [485, 1820]	1110 (325) [560, 2120]	1126 (343) [400, 2120]	1155 (343) [560, 2120]
Birth weight z score	-1.47 (1.48) [-4.70, 2.20]	-1.53 (1.51) [-4.70, 2.20]	-1.36 (1.49) [-4.90, 3.40]	-1.44 (1.47) [-4.70, 2.20]
Birth weight z score < -2,0	39 (32 %)	36 (34 %)	54 (31 %)	38 (28 %)
Apgar at 5 min < 5	19 (16 %)	13 (12 %)	30 (17 %)	17 (13 %)
Clinical chorioamnionitis	35 (29 %)	34 (32 %)	55 (31 %)	39 (29 %)
Chronic lung disease	18 (15 %)	14 (13 %)	23 (13 %)	19 (14 %)
Necrotising enterocolitis	3 (2 %)	3 (3 %)	11 (6 %)	3 (2 %)
Septicaemia	16 (13 %)	11 (10 %)	30 (17 %)	12 (9 %)
Neonatal death	7 (6 %)	0 (0 %)	0 (0 %)	0 (0 %)

4.2.2 Histological analyses of the placenta (I, II, IV)

The placenta was routinely inspected by a midwife or an obstetrician after birth and thereafter immediately immersed in 10% aqueous solution of formalin for fixation. The size, weight, insertion of umbilical cord, and possible gross pathological features were recorded. A pathologist examined the placenta, the membranes, and the umbilical cord and sampled tissue for the histological process. At least two non-consecutive sections were studied from every placenta, umbilical cord and fetal membranes. In the case of gemini or trigemini pregnancies, all placentas, cords and membranes were sampled. Extra samples were taken if warranted by macroscopical impression. The histological specimens were prepared in a routine manner through an ascending series of ethanol, xylene, embedded in paraffin, cut at 3-5 μm and rehydrated in a series of xylene and a descending series of ethanol and stained with hematoxylin and eosin.

All specimens were analysed by two investigators (HK, MR) blinded to clinical and neuroradiological findings. Chorioamnionitis was divided into maternal and fetal components, and each case was assigned a stage and a grade as described by Redline et al. (Redline et al., 1998) (Table 4). Consensus scores were used for grading and staging. In addition, other findings such as the presence of hemangiomas of the umbilical cord, foci of calcification, and infarcts were recorded.

TABLE 4. Chorioamnionitis was designated into maternal and fetal components, and each case was assigned a stage and a grade (Redline et al., 1998).

Maternal stages	
1	Neutrophils in subchorionic fibrin of chorionic plate
2	Neutrophils in the chorion laevae
3	Neutrophils in the chorion laevae and adjacent amnion
4	Neutrophil karyorhexis or eosinophilia of basement membrane without amnionic epithelial sloughage
5	Neutrophils plus amnionic epithelial sloughage

Maternal grades*	
1	Rare neutrophils (<10/high power field [HPF])
2	Intermediate neutrophils (11 – 30/HPF)
3	Abundant neutrophils (>30/HPF)

*Maternal grade was based on intensity of inflammation in the area worst affected.

Fetal stages	
1	Neutrophils in chorionic vessels only
2	Neutrophils in the umbilical cord vein wall (umbilical phlebitis)
3	Neutrophils in all 3 umbilical vessel walls (umbilical panvasculitis)
4	Neutrophils in Wharton's jelly (umbilical perivasculitis)
5	Subnecrotizing funisitis

Fetal grades*	
1	Low
2	Mediate
3	High

*Fetal grade was based on the intensity of inflammation in the most severely affected umbilical or chorionic plate vessel.

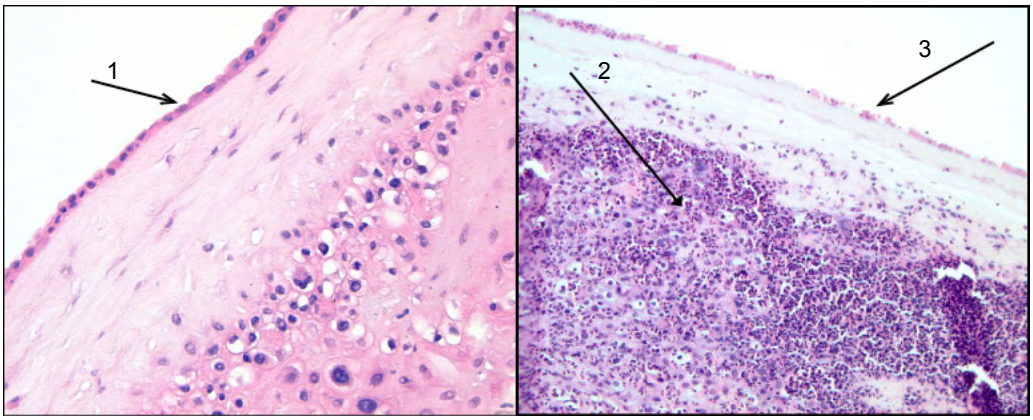

FIGURE 2. On the left, normal fetal membrane with intact amnion epithelium (1). On the right, severe chorioamnionitis with dense infiltrate of granulocytes (2) and sloughing of amnion epithelium (3).

4.2.3 IL-6 genotyping (II, III)

DNA was extracted from EDTA blood samples using the nucleon BACC3-reagent kit (Amersham Biosciences, US). Analyzed IL-6 promoter regions were amplified using polymerase chain reaction (PCR). The primers were chosen on the basis of previous published studies (Brull et al., 2001; Fishman et al., 1998). PCR was performed in 25 µl using 100 ng genomic DNA as a template, 0.4 µM of each primer, 80 µM dNTP and ~2 U DNA polymerase [DynaZyme II/DynaZyme EXT (Finnzymes, FIN) for IL-6 -572 and FastStart Taq (Roche Diagnostics, GE) for IL-6 -174]. PCR included 5 min denaturation at 95ºC and 35 amplification cycles (40 s at 95ºC, 30 s at 60ºC and 40 s at 72ºC for IL-6 -572 and 1 min at 95ºC, 1 min at 53ºC and 1 min at 72ºC for IL-6 -174) and a final elongation of 5 min at 72ºC. Genotypes were resolved by using restriction fragment length polymorphism. The restriction endonucleases used were BsrBI (NEB, US) for IL-6 -572 and NIaIII (NEB, US) for IL-6 -174. Digestion was performed at 37ºC in 15 µl using 6-10 µl of PCR product and 3 U of restriction endonuclease for 2 h. The size of the digestion products was determined by using agarose gel electrophoresis.

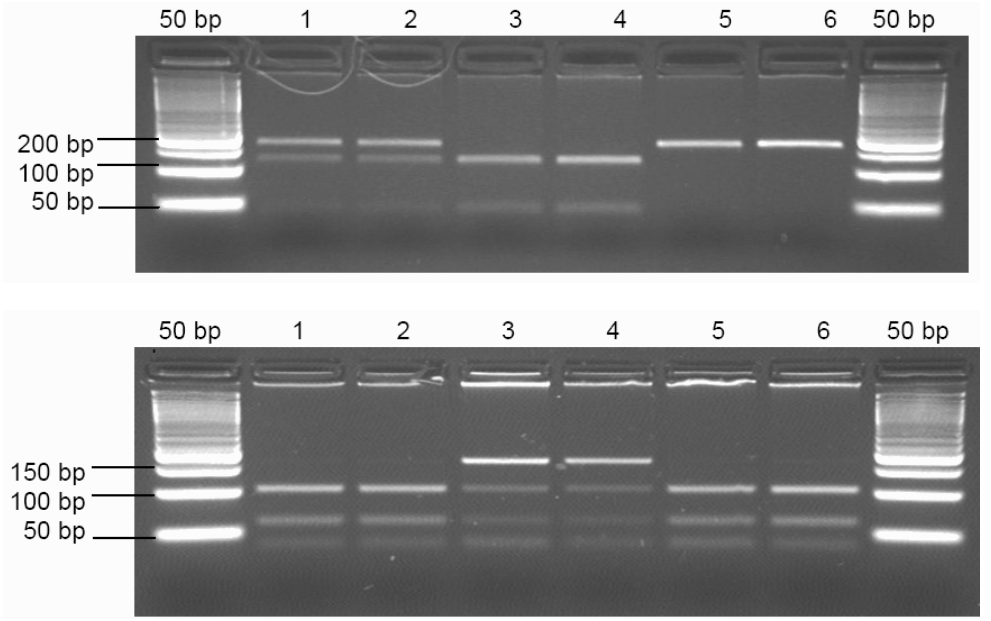

FIGURE 3. IL-6 -174 (above) and -572 (below) single nucleotide polymorphism restriction fragment analyses. On the sides, 50 base pair molecule weight markers. Above, 1 and 2; -174 GC restriction fragments, 3 and 4; -174 CC restriction fragments, 5 and 6; -174 GG restriction fragments. Below, 1, 2, 5, and 6; -572 GG restriction fragments, 3 and 4; -572 GC restriction fragments.

4.2.4 Cranial ultrasound examination (I, III)

Cranial US examinations in the neonatal intensive care unit were performed for all study infants at 3 to 5 days, at 7 to10 days, at one month of age and, thereafter, monthly until discharge from hospital. The US examinations were performed using a 7 MHz vector transducer (Sonos 5500 Hewlett-Packard). The classification of IVH (grades I to IV) was done according to Papile (Papile et al., 1978). Multiple cysts with typical location were classified as cystic PVL.

The cranial US examination at term was performed with a 7.5 MHz vector transducer (Aloka SSD 2000) during 1/2002 – 8/2002 and an 8 MHz vector transducer (General Electric Logic 9) thereafter by a pediatric radiologist (HR). Ventriculomegaly was defined according to the reference values for VLBW infants at term (Virkola 1988).

The infants were categorized into three groups according to the most pathological finding on brain US examinations: 1) normal, 2) mildly abnormal and 3) severely abnormal. The division into these groups was done as described by Rademaker et al. (Rademaker et al., 2005) (Table 5).

TABLE 5. Cranial US classification (Rademaker et al., 2005).

Normal	No abnormalities
	Germinal layer/plexus cysts
	Subependymal pseudocysts
	Calcifications
Mildly abnormal	IVH grade I/II
	Germinal layer necrosis
	Ventricular dilatation
Severely abnormal	IVH grade III/IV
	Cystic PVL II/III
	Thalamic lesion
	Focal infarction
	Convexity haemorrhage
	Ventricular dilatation following a haemorrhage with need for therapeutic intervention

4.2.5 Brain MRI (I, III, IV)

MRI study of the brain was performed at term on the same day as the US examination. The imaging took place during postprandial sleep without sedation or anesthesia. Ear protection was used (3M Disposable Ear Plugs 1100, 3M, Brazil; Würth Hearing protector, Art.-Nr. 899 300 232, Würth, Austria). The MRI equipment was either an open 0.23 Tesla Outlook GP (Philips Medical Inc., Vantaa, Finland), or 1.5 Tesla Philips Intera (Philips Medical Systems, Best, The Netherlands). The information about brain lesions in MRI was not used for further analysis as the upgrading of the equipment may result in non-comparable findings between the first and the latter half of the sample.

4.2.5.1 Regional volumes (I, III)

For volume measurements, at 0.23 T we obtained a T1-weighted field echo sequence with time repetition (TR) of 30 ms, a time echo (TE) of 10 ms, a flip angle of 45 degrees, a slice thickness of 5 mm, a field of view of 220 x 220 mm^2 and a matrix of 256 x 256 was obtained in the coronal plane. At 1.5 T we obtained coronal T1-weighted inversion recovery sequence TR of 3500 ms, a TE of 400 ms, a time inversion of 15 ms, a flip angle of 90 degrees, a slice thickness of 4.8 mm, a field of view (FOV) of 180 x 180 mm^2 and a matrix of 256 x 256. The sequences were optimized relative to the field strength of the equipment used.

The postacquisition volume measurements were performed on a GE workstation (GE AW1.0, GE Medical Systems, Milwaukee, USA) by one neuroradiologist (RP). The coronal T1-weighted images were loaded in the Functool 1.0.post-processing soft-ware (GE Medical Systems, Milwaukee, USA). The volume measurement was manually performed separating the cerebrospinal fluid and the skull from brain tissue image by

image. Anatomical differentiation of the brain areas was based both on the anatomic landmarks and on signal intensity differences of the brain structures. The regional brain volumes measured were cerebral volume, cerebellar volume, frontal lobe volume, the combined volume of the medulla oblongata and the pons and the combined volume of basal ganglia and thalami. The basal ganglia and thalami were measured as a block and the anatomic border between these basal gray matter nuclei and unmyelinated deep white matter on both field strength images was easily delineated by visual inspection. The medial border of the basal ganglia and thalami was formed by the third ventricle, the lateral border was formed by the external capsule and the inferior border was formed by the upper border of the mesencephalon.

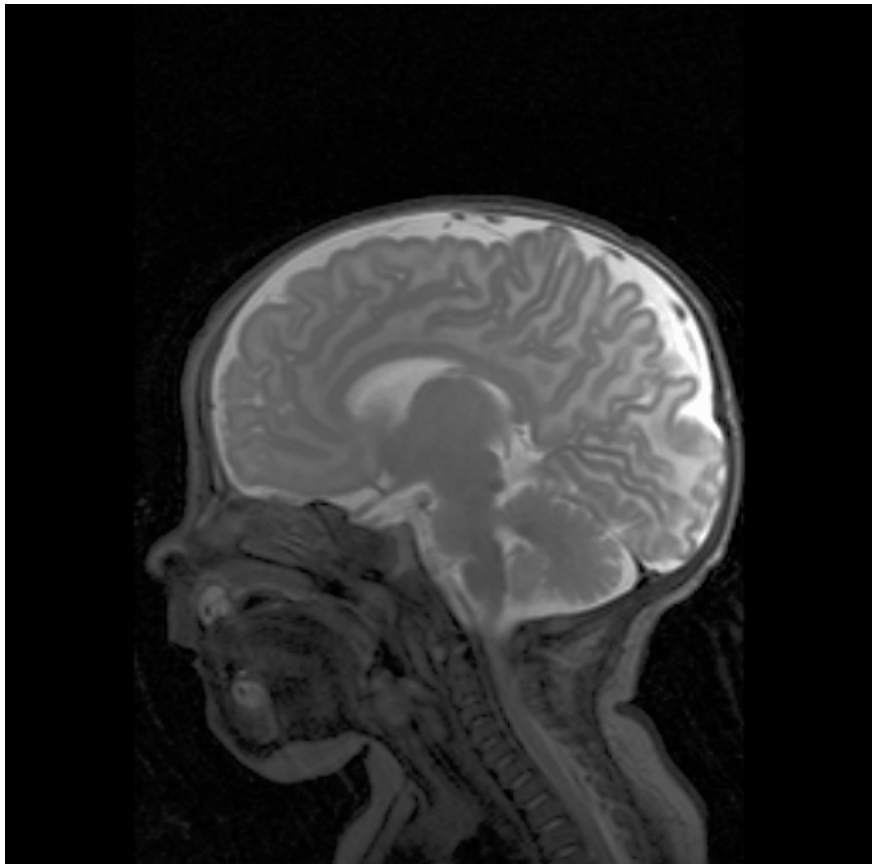

FIGURE 4. T2 weighted midline magnetic resonance image of a preterm infant's brain at term age.

4.2.5.2 Diffusion tensor imaging (IV)

The infants were imaged using 1.5 T high field strength MR equipment at term age. The infants were imaged with the following protocol: axial T2-weighted images with TR of

5097 ms, a TE of 120 ms, a flip angle of 90 º, a slice thickness of 4 mm, a FOV 200 x 200 mm2 and a matrix of 256 x 256, axial T1-weighted images with a TR of 496 ms, a TE of 14 ms, a flip angle of 90º, a slice thickness of 4 mm, a FOV of 200 x 200 mm2 and matrix of 256 x 256, sagittal T2-weighted images with a TR of 4851 ms, a TE of 120 ms, a flip angle of 90º, a slice thickness of 4 mm, a FOV of 200 x 200 mm2 and a matrix of 256 x 203.

The diffusion tensor imaging was performed with the following sequence: the TR was 2837 ms, the TE was 68 ms, slice thickness was 5 mm, the FOV was 200 x 200 mm^2 and the reconstructed matrix was 256 x 256. We acquired a T2-weighted reference image (b=0 s/mm^2) and 15 diffusion weighted images (b=600 s/mm^2) in noncollinear gradient directions per section. We used a lower b-value than in the imaging of the adults, because of the immaturity and higher water content of the brain of infants compared to that of adults. The FA values and the ADC values of the right and left inferior colliculus were measured twice by a neuroradiologist (RP) using Pride software (Philips Medical Systems, Best, The Netherlands) with the region of interest (ROI) method. The pixel size in diffusion tensor images was 0.78 x 0.78 mm x mm. The mean ROI area in FA and ADC measurements was 28.6 pixels (SD 5.7 pixels) and ranges 16 to 41 pixels. The reproducibility of the ROI measurements was verified by two measurements. The Pearson´s correlation coefficient of repeated ROI measurements for FA in the right colliculus inferior was 0.74819, for FA in the left colliculus inferior it was 0.80582, for ADC of the right colliculus inferior it was 0.42177 and for the ADC of the left colliculus inferior it was 0.72675. All of the p values were significant ($p<0.0001$, $p<0.0001$, $p<0.0006$ and $p<0.0001$, respectively).

4.2.6 Brainstem auditory-evoked potential (IV)

The BAEP recordings were carried out according to the routine clinical BAEP recording procedure at the department of clinical neurophysiology, Turku University Hospital. Median age for BAEP recording was 30 days after term age (range -23-202 days from term age). The laboratory was soundproof and fully equipped for clinical evoked potential measurements. The room temperature was kept at 20-22ºC. Prior to the BAEP recording, a pediatrician inspected the auditory canals with an otoscope for possible obstacles (ear wax was removed) and middle ear infections. In case of an infection, BAEP recording was rescheduled. During the recording, the participant lay on a bed or in the parent's arms. A nurse inserted tubal insert phone electrodes (TIPtrode; Nicolet Biomedical Instruments, Madison, WI, USA) that delivered auditory stimuli. The BAEP responses were recorded with surface electrodes on both mastoid processes. The forehead was carefully wiped with alcohol-soaked gauze and three Ag-AgCl electrodes were attached on the skin, the ground at midline, and the two reference electrodes for both sides at the Fp1' and Fp2'

positions according to the international 10/20 system of electrode placement. Electrode impedances were kept under 5.0 kΩ.

The BAEP recording was performed with an eight channel Nicolet Viking IV device (Nicolet Biomedical Instruments, Madison, WI, USA). The BAEPs were elicited with a rarefaction click stimulus of 85 dB nHL intensity and 100 μs duration given at a rate of 10.3 Hz, while a masking noise of 45 dB nHL was delivered to the contralateral ear, and the responses were amplified with the high and low pass filters set at 100 Hz and 3 kHz, respectively. For both sides, the BAEP was recorded at least twice to ascertain reproducibility. The peak latencies of waves I, III, and V, as well as the interwave intervals (I-III, I-V, III-V) were measured from ipsilateral responses on both sides. The amplitudes of wave I and V were measured from the negative peak to the following through (I´, V´), and the amplitude ratio I/V was also calculated. Waves I, III, and V were identified and marked manually on the computer screen by a nurse, and the results were confirmed and visually analyzed by a clinical neurophysiologist (SJ).

4.2.7 Statistical analyses

In all studies, diagnostic plots were produced to ensure that assumptions of the analyses were reasonably satisfied. If assumptions of parametric analyses were not met, *variables were transformed to achieve* approximate normal distributions.

In studies I-III, univariate analyses were performed using the following methods:

The effect of categorical predictor variables on continuous outcome variables was studied using One-way analysis of variance. Association between continuous predictor variables and brain volumes were studied using regression analysis. Group comparisons between study infants and the VLBW infants excluded due to missing placental samples were assessed with Pearson's Chi-Square test for categorical variables. Logistic regression analysis was used to determine the relationship between IL-6-174 and -572 genotypes (GG/GC/CC) and categorical outcome variables. If the logistic regression model did not converge, significance levels were obtained using Fisher's exact test. Cumulative logit models were created to test the hypothesis that explanatory variables were associated with ordinal brain ultrasound findings.

In study IV, univariate associations between continuous variables were analysed using Pearson's correlation coefficients separately on both the right and left side. The paired-samples t-test was used to measure if there was any difference in the results of the BAEP and DTI measurements between the left and right sides. Associations between categorical independent variables and continuous outcome variables were studied using mixed model repeated measures analyses. To correct for a possible correlation between two

observations from one child, compound symmetry was used as a correlation structure in all mixed models.

In study I, post-hoc power analysis with 80% power was used to calculate the detectable mean difference in total brain volumes between the cases with and without placental inflammation.

In study I, multivariate analysis was done using stepwise methods. Cumulative logit models were used for ordinal outcome variables and multiple regression was used for continuous outcome variables. Variables significant at p-value below 0.05 were retained in the final stepwise model. Analysis of covariance was used to study the effect of brain pathology on brain volumes controlling for the same covariates as in the final model of the previous multiple regression analysis.

In studies II and III, analysis of covariance was used for multivariate analysis of the effects of IL-6-174 and -572 genotypes on continuous outcome variables, controlling for any confounding effects of gender, gestational age and the z score of birth weight. Associations between IL-6-174 and -572 genotypes and dichotomous outcome variables were further studied using logistic regression models, which were adjusted for the same variables as above. Cumulative logit models were used for ordinal variables, again adjusted for the same variables. When gestational age was used as an outcome variable, only the z score of birth weight and gender were used as covariates.

In study IV, the first stage of the multivariate analysis of each outcome variable was to enter within subjects variable side, between subjects background variables gender, gestational age, birth weight SD, and age at BAEP measurement to a mixed model as fixed effects. Age at BAEP recording was not used as a predictor of FA and ADC values. Then histological chorioamnionitis, fetal chorioamnionitis, combined CLD and NEC, and septicaemia were added to the mixed model one at a time and their effects were assessed in the presence of background variables.

In all studies, statistical analyses were performed using SAS (version 9.1; SAS Institute, Cary, NC). Results are presented with 95 % confidence intervals. Two-sided p values were used with $p<0.05$ being considered statistically significant.

5. RESULTS

5.1. Placental pathology (I, II, III)

In our study population, the prevalence of maternal chorioamnionitis was 44 %, fetal chorioamnionitis 23-26 %, and total prevalence of histological chorioamnionitis was 40-45 % (Table 6). The statistical analyses were done with dichotomization of histological chorioamnionitis, without using detailed information on stages and grades, as this was found not to affect the results.

In study I, in the presence of clinical chorioamnionitis, histological inflammation was found in 94% of the placentas. On the other hand, clinical chorioamnionitis was present in 61 % of cases with maternal histological chorioamnionitis and in 81 % of the cases with fetal chorioamnionitis. There were only 7 cases with PROM alone without other signs of clinical chorioamnionitis or histological chorioamnionitis.

TABLE 6. The prevalence of histological chorioamnionitis in the study population.

	Study I n=121	Study II n=107	Study IV n=135
Histological chorioamnionitis	54 (45 %)	47 (44 %)	49 (40 %)
Maternal chorioamnionitis	53 (44 %)	-	-
Fetal chorioamnionitis	31 (26 %)	-	28 (23 %)

5.2. IL-6 polymorhism (II, III)

The prevalence of different genotypes did not differ significantly from the Finnish reference population (Hulkkonen et al., 2001; Noponen-Hietala et al., 2005). The prevalence of IL-6 -174 and -572 genotypes (GG/GC/CC) is shown in Table 7. In study II, IL-6 -174 genotyping was done successfully to all but one, and -572 genotyping was done successfully to all of the study infants. In study III (n=175), IL-6 -174 and -572 genotyping was done successfully on all but one sample in both groups.

TABLE 7. The prevalence of IL-6 -174 and 572 genotypes (GG/GC/CC) in the study population.

	Study II n=107			Study III n=174		
	GG n (%)	GC n (%)	CC n (%)	GG n (%)	GC n (%)	CC n (%)
Il-6 -174	29 (27 %)	53 (50 %)	24 (23 %)	38 (22 %)	91 (52 %)	45 (26 %)
IL-6 -572	102 (95 %)	5 (5 %)	0 (0%)	163 (94 %)	11 (6 %)	0 (0 %)

5.3. Brain lesions (I, III)

Cranial US examinations were carried out according to the protocol in the intensive care unit, and infants were categorized into three groups according to the most pathological finding on brain ultrasound examinations. Out of the 121 study patients in study I, 7 died before term. All of them were classified into the severely abnormal brain pathology group. Five of them had confirmed brain pathology at autopsy. Study III did not include non surviving infants. The findings in brain US examinations are shown in Table 8.

TABLE 8. Findings from the cranial ultrasound examinations of study infants until term age.

	Study I n=121	Study III n=175
Normal	56 (45 %)	97 (55 %)
Mildly abnormal	50 (40 %)	66 (38 %)
Severely abnormal	19 (15 %)	12 (7 %)

5.4. Regional brain volumes (I, III)

In study I, 103 infants of the 113 surviving study infants were successfully measured for regional brain volumes. One infant missed MRI because of being in another hospital at term age, and nine infants had qualitatively inadequate images preventing reliable volume measurements. In study III, 153 study infants were measured for regional brain volumes. Of the 175 study patients, 3 infants missed brain MRI and 19 had qualitatively inadequate images preventing reliable volume measurements. Mean values of the regional brain volumes are shown in Table 9.

TABLE 9. Mean values (ml) and [SD] of the regional brain volumes in the study population.

	Study I n=103	Study III n=153
Cerebrum	370.4 [45.4]	369.7 [48.0]
Cerebellum	24.4 [4.4]	24.2 [5.3]
Frontal lobe	128.9 [23.9]	126.4 [24.8]
Medulla oblongata and pons together	6.0 [2.39]	6.0 [2.3]
Basal ganglia and thalami together	24.9 [4.6]	25.1 [4.5]

5.5. Diffusion tensor imaging of the inferior colliculus (IV)

DTI studies were performed at term age successfully on 59 of the study infants. The low number of DTI measurements is due to the lack of 1.5 T MRI studies prior to June 2004, and to some qualitatively inadequate images. Results from the DTI measurements are shown in Table 10.

TABLE 10. Means, (SD) and [min, max] presented for fractional anisotropy (FA) and apparent diffusion coefficient (ADC) of inferior colliculus in diffusion tensor imaging (DTI). Results of DTI studies of inferior colliculus are available from 59 study infants. Values are shown separately for right and left side (p-value and SD are shown for comparison).

DTI	Right	Left	p (SD)
FA	0.361 (0.055) [0.235, 0.496]	0.370 (0.054) [0.236, 0.513]	0.067 (0.04)
ADC	1.065 (0.115) [0.879, 1.427]	1.013 (0.084) [0.864, 1.221]	0.001 (0.12)

5.6. Brainstem auditory evoked potentials (IV)

Altogether, 129 infants had BAEP recordings done. Eight infants had some or all of the BAEP waves absent. The absent responses could not be used in the statistical analyses of latencies. Results from the BAEP recordings are shown in Table 11.

TABLE 11. Means, (SD) and [min, max] presented for brainstem auditory evoked potential (BAEP) values (latencies (I, III, V), inter peak latencies (I-III, I-V, III-V), amplitudes (I-I', V-V') and amplitude ratio (I/V)). Results of BAEP recordings are shown of 129 study infants. Values are shown separately for right and left side (p-value and SD are shown for comparison). The distribution of absent responses is also shown.

BAEP*	Right	Left	p (SD)
I (ms)	2.07 (0.14) [1.68, 2.76]	2.09 (0.16) [1.62, 2.88]	0.179 (0.11)
III (ms)	4.80 (0.31) [4.11, 6.06]	4.82 (0.29) [4.32, 5.79]	0.406 (0.21)
V (ms)	7.01 (0.34) [6.03, 8.25]	7.00 (0.32) [6.24, 7.80]	0.282 (0.18)
I-III (ms)	2.70 (0.26) [1.98, 3.87]	2.72 (0.25) [2.28, 3.60]	0.430 (0.18)
I-V (ms)	4.93 (0.31) [4.08, 6.06]	4.90 (0.30) [4.08, 5.61]	0.038 (0.16)
III-V (ms)	2.21 (0.27) [1.11, 2.88]	2.18 (0.25) [1.23, 2.73]	0.048 (0.20)
I-I' (μV)	0.41 (0.25) [0, 1.21]	0.41 (0.22) [0, 1.01]	0.805 (0.28)
V-V' (μV)	0.36 (0.16) [0, 0.95]	0.37 (0.17) [0, 0.94]	0.351 (0.19)
I/V	1.33 (1.03) [0, 5.13]	1.44 (1.69) [0, 14.5]	0.733 (0.58)
Absent responses	n=8	n=4	
I	5	2	
III	5	3	
V	5	3	

5.7. Histological chorioamnionitis in association with brain lesions (I)

Histological chorioamnionitis on the fetal side was associated with brain lesions in univariate analysis (p= 0.023, OR= 2.46, CI= 1.13– 5.41), whereas overall histological chorioamnionitis (including maternal and fetal chorioamnionitis) and maternal chorioamnionitis were not. In addition, clinical chorioamnionitis (p= 0.016, OR= 2.54, CI= 1.19– 5.40), low gestational age (p= <0.0001, OR= 1.06, CI= 1.03– 1.08 per one day decrement), low birth weight z score (p= 0.050, OR= 1.27, CI= 1.00– 1.61 per one unit increase) and low Apgar scores (p= 0.002, OR= 4.52, CI= 1.73– 11.82) associated with brain lesions in univariate analyses.

In multivariate analyses using stepwise cumulative logistic regression analysis, the only significant background factor associating with brain lesions was the infant's low gestational age ($p<0.0001$, OR= 1.06, CI= 1.03– 1.08 per one day decrement).

5.8. Histological chorioamnionitis in association with regional brain volumes (I)

Histological and clinical chorioamnionitis were not associated with regional brain volumes.

Female gender and lower z score of birth weight correlated with smaller total brain tissue volume in multivariate analyses using a stepwise multiple regression model ($p= 0.001$ and $p<.0001$, respectively). In addition, lower gestational age associated with smaller total brain volume ($p= 0.0002$).

Smaller cerebellar volumes associated with female gender ($p= 0.047$), low birth weight z score ($p=0.001$), and low gestational age ($p= 0.003$) in multivariate analyses.

The combined volume of the basal ganglia and thalami was associated with a low birth weigh z score in multivariate analyses ($p= 0.0002$). In addition, low gestational age was a risk factor for small combined volume of basal ganglia and thalami ($p= 0.007$).

None of the background variables were associated with the combined volume of medulla oblongata and pons.

5.9. Histological chorioamnionitis and neonatal infections in association with BAEP measurements (IV) (unpublished data)

Of the 129 infants with BAEP recordings, 117 had available placental samples. Histological chorioamnionitis (including maternal and fetal chorioamnionitis) was not associated with BAEP responses, but fetal chorioamnionitis associated with prolongation of the III-V interval in univariate analyses (estimate=0.175, SE=0.052, p=0.001). Combined CLD and/or NEC were associated with the prolongation of wave V latency (estimate=0.192, SE=0.080, p=0.019), and septicaemia associated with the prolongation of wave III latency (estimate=0.224, SE=0.095, p=0.020) and the diminishing of wave I amplitude (estimate=-0.138, SE=0.055, p=0.014) and I/V amplitude ratio (estimate= -0.277, SE=0.114, p=0.017). In multivariate analysis controlled for previously entered variables (side of measurement, gender, gestational age and z score of birth weight and age at the time of BAEP examination), fetal chorioamnionitis no longer associated with BAEP results. The associations between combined CLD and/or NEC and wave V latency (estimate=0.17, SE=0.08, p=0.044), and septicaemia and wave III latency (estimate=0.21, SE=0.10, p=0.037) were still seen in multivariate analyses, but the effect of septicaemia on wave I amplitude was no longer significant.

5.10. Histological chorioamnionitis and neonatal infections in association with the DTI of the inferior colliculus (IV) (unpublished data)

Forty-six infants had both DTI and placental samples available. Histological chorioamnionitis and neonatal inflammatory diseases had no association with FA and ADC of the inferior colliculus in univariate analyses or in multivariate analyses controlled for previously entered variables (side of measurement, gender, gestational age and z score of birth weight and age at the time of BAEP examination). In addition, gender, gestational age, and z score of birth weight were not associated with FA and ADC of the inferior colliculus.

5.11. Correlation between the BAEP and DTI of the inferior colliculus (IV)

Fifty six infants had both BAEP recording and DTI done. Wave I, III, and V latencies were negatively correlated with FA values of thr inferior colliculus on the left side (correlation coefficient r=-0.28, p=0.038; r=-0.27, p=0.046; r=-0.30, p=0.027 respectively). In addition, wave I latency correlated positively with ADC value on the left (r=0.29, p=0.033). I-III interval correlated negatively with FA values on the right side (r=-0.37, p=0.008). Wave V amplitude was positively correlated with FA values on the left side (r=0.27, p=0.047).

5.12. IL-6 polymorphism in association with histological chorioamnionitis (II)

The IL-6-174 GG genotype was associated with histological chorioamnionitis both in univariate (p=0.023, GG versus CC: OR=3.80, CI=1.21–11.92; GG versus GC: OR=3.40, CI =1.32–8.79; GC versus CC: OR=1.12, CI=0.40–3.09) and in multivariate analyses adjusted for gender, gestational age and z score of birth weight (p=0.009, GG versus CC: OR=6.31, CI=1.51–26.43; GG versus GC: OR=5.56, CI =1.73–17.85; GC versus CC: OR=1.14, CI=0.33–3.87). In addition, the IL-6 -572 GC genotype correlated to histological chorioamnionitis in multivariate analyses (p=0.039, OR=16.22, CI=1.15–228.43). IL-6 -174 or -572 genotypes were not associated with gestational age.

5.13. IL-6 polymorphism in association with neonatal infections (II)

Of the eleven study infants with septicaemia, the majority had septicaemia caused by Gram-positive bacteria (n=10), including coagulase negative Staphylococci (CONS) and group B Streptococci. One septicaemia was caused by Gram-negative bacteria (*Klebsiella pneumoniae*) and one fungal infection occurred in a patient with previous CONS-septicaemia. Since only 3 infants had NEC, it was grouped together with CLD for statistical analyses.

IL-6-174 genotypes correlated to septicaemia both in univariate analyses (p=0.012, CC versus GG: OR=3.57, CI=0.81–15.74; GC versus GG: OR=0.17, CI=0.02–1.68; CC versus GC: OR=21.41, CI=2.46–186.72) and in multivariate analyses adjusted for gender, gestational age and z score of birth weight (p=0.027, CC versus GG: OR=3.05, CI=0.65–14.36; GC versus GG: OR=0.17, CI=0.02–1.78; CC versus GC: OR=18.27, CI=1.98–168.37). From the 11 infants with septicaemia, 7 had IL-6 -174 CC genotype, and they all had Gram-positive infections. IL-6 -174 genotypes were not associated with CLD and/or NEC. IL-6-572 genotypes were not associated with septicaemia or CLD and/or NEC in univariate analyses. Multivariate analyses could not be done, because none of the infants with the -572 GC genotype had septicaemia, CLD, or NEC.

5.14. IL-6 polymorphism in association with brain injury (III)

IL-6 -174 and -572 genotypes were not associated with the incidence of mildly or severely abnormal brain lesions in univariate or in multivariate analyses adjusted for gender, gestational age and z score of birth weight. Only gestational age associated significantly with structural brain lesions in US examinations (p<0.0001, OR=1.04, CI=1.02–1.06).

5.15. IL-6 polymorphism in association with regional brain volumes (III)

Mean values of the regional brain volumes related to the IL-6 -174 and -572 genotypes are shown in Table 12.

TABLE 12. Mean values (ml) and [95 % confidence intervals] of the regional brain volumes related to the IL-6 -174 and -572 genotypes (GG/GC/CC) in the study population (n=153).

	Cerebrum	Cerebellum	Frontal lobe	Medulla oblongata and pons together	Basal ganglia and thalami together
IL-6-174					
CC	361.6 [346.0 – 377.2]	23.4 [21.7 – 25.1]	123.2 [115.0 – 131.3]	5.1 [4.6 – 5.8]	23.5 [22.0 – 24.9]
GC	372.2 [361.8 – 382.6]	24.0 [22.8 – 25.1]	126.6 [121.2 – 132.0]	5.3 [4.9 – 5.6]	25.2 [24.3 – 26.2]
GG	375.1 [359.5 – 390.7]	25.8 [24.1 – 27.5]	130.5 [122.4 – 138.6]	5.9 [5.3 – 6.6]	26.7 [25.2 – 28.1]
IL-6-572					
GG	368.4 [360.5 – 376.3]	24.0 [23.1 – 24.8]	125.4 [121.3 – 129.4]	5.3 [5.0 – 5.6]	24.8 [24.1 – 25.6]
GC	380.8 [350.9 – 410.7]	26.2 [22.9 – 29.5]	140.2 [124.8 – 155.6]	5.9 [4.9 – 7.5]	28.7 [25.9 – 31.5]

The IL-6 -174 CC genotype associated with a smaller volume of basal ganglia and thalami both in univariate (p= 0.008, CC versus GC: estimated difference between means= -1.77, CI= -3.82– 0.27; CC versus GG: estimated difference between means= -3.19, CI= -5.61– -0.78; GC versus GG: estimated difference between means= -1.42, CI= -3.47– 0.63) and in multivariate analyses controlling for possible confounding effects of gender, gestational age and z score of birth weight (p=0.009, CC versus GC: estimated difference between means= -1.34, CI= -3.30–0.61; CC versus GG: estimated difference between means= -3.03, CI= -5.33– -0.73; GC versus GG: estimated difference between means= -1.69, CI= -3.64– 0.27). The IL-6 -572 GG genotype also associated with a smaller volume of basal ganglia and thalami both in univariate (GG versus GC: p= 0.008, estimated difference between means= -3.86, CI= -6.72– -1.00) and in multivariate analyses (GG versus GC: p=0.009, estimated difference between means= -3.60, CI= -6.30– -0.91). In addition, smaller gestational age and smaller z score of birth weight were significantly associated with reduction in basal ganglia and thalami volume (p=0.004, estimated difference between means=0.06, CI=0.02 – 0.10; p<0.0001 estimated difference between means=1.22, CI=0.70 – 1.74 respectively). IL-6-174 and -572 genotypes were not associated with the other regional brain volumes measured.

The associations between IL-6 -174 genotypes and basal ganglia and thalami volume was not explained by the association between the same genotype and septicaemia (unpublished data).

6. DISCUSSION

The PIPARI Study was designed to investigate the brain development in the context of preterm birth focusing on the risk factors and protective factors beginning from the fetal life. The outcome measures included brain injuries as detected by imaging methods and the developmental outcome of the child. This part of the PIPARI Study focuses on the role of antenatal inflammation on the brain imaging findings.

Despite the fact that chorioamnionitis has been widely studied in association with preterm infants' brain pathology, this is the first study to evaluate the effects of histological chorioamnionitis and IL-6 promoter polymorphisms on brain pathology in an unselected VLBW/VLGA infant cohort using modern imaging equipment, including volumetric MRI as well as DTI.

6.1. Histological chorioamnionitis in association with brain lesions

Our findings are in contrast with several earlier studies (DiSalvo, 1998; Kaukola et al., 2006; Leviton et al., 1999; Polam et al., 2005; Vergani et al., 2000) which suggested that chorioamnionitis plays a major role in brain pathology in VLBW/VLGA infants. We found no independent role for histological chorioamnionitis on the brain pathology of VLBW/VLGA infants. Similar to our results, several studies consisting of preterm infants born before 34 weeks of gestation have concluded that there is no evidence of chorioamnionitis directly associating to brain injuries (Kumazaki et al., 2002; Murata et al., 2005; Vergani et al., 2004). Andrews et al. (Andrews et al., 2008) even reported a trend indicating higher IQ in the presence on funisitis in VLBW infants. As the only independent risk factor for brain pathology in this study population of VLBW/VLGA infants was low gestational age, it is plausible that the association between histological chorioamnionitis and brain pathology reflects the strong link between chorioamnionitis and preterm birth, which, itself, relates to brain pathology. It is also worth noting that even though there is strong evidence associating chorioamnionitis with brain pathology and abnormal neurological development in term infants, data concerning preterm infants is scarce. As there is always some pathology underlying a preterm birth, our results can be interpreted so that histological chorioamnionitis, even though possibly affecting the brain, does not cause any greater risk for brain pathology than other causes behind preterm delivery.

Earlier studies have suggested fetal inflammation to be more deleterious to the preterm infants' central nervous system than inflammation limited to the maternal tissues (Bejar et al., 1988; DiSalvo, 1998; Leviton et al., 1999; Yoon et al., 2003). This hypothesis was also supported by our study, as fetal chorioamnionitis associated with brain injury in univariate analysis even though the association was not seen after adjusting for

confounding factors. The deleterious effects of fetal inflammatory response may be caused by elevated cytokine levels. It has been shown in term placentas that cytokines do not pass the placenta (Aaltonen et al., 2005), thus preventing the matermal cytokines affecting the fetuses' brain.

6.2. Histological chorioamnionitis in association with regional brain volumes

VLBW/VLGA infants (Inder et al., 2005; Shah et al., 2006; Thompson et al., 2007), and even preterm infants born closer to term age (Boardman et al., 2006; Limperopoulos et al., 2005; Peterson et al., 2003; Srinivasan et al., 2007), have been shown to have smaller regional brain volumes at term age compared with term born infants. The etiology of reduced brain volumes is not yet understood in detail, but it has been suggested that premature birth alone does not sufficiently explain small regional brain volumes at term age in preterm infants (Boardman et al., 2007; Mewes et al., 2006) or in the VLBW/VLGA sub group (Thompson et al., 2008). Our hypothesis was that histological chorioamnionitis is one of the factors leading to reduced brain volumes but this was not supported by our results. As far as we know, this was the first study to evaluate the association between placental inflammation and brain volumes. According to our study, it seems that low gestational age and delayed growth for gestational age are the best predictive factors for small brain volumes in VLBW/VLGA infants. They were both independent risk factors for all but one regional brain volume i.e. the combined volume of the medulla oblongata, and pons. The lack of association with brain stem volume is possibly due to the early fetal development of this area, making it less prone to developmental problems caused by the insult of preterm delivery (Cohen et al. 1990).

Severely abnormal brain findings were associated with several smaller regional brain volumes in our study. This finding is also in accordance with earlier studies associating brain pathology with reduced brain volumes in VLBW/VLGA infants (Inder et al., 2005; Inder et al., 1999; Lin et al., 2001; Thompson et al., 2008; Thompson et al., 2007; Vasileiadis et al., 2004). Considering that low gestational age had a strong association with brain lesions, it may be that brain lesions explain the association seen between low gestational age and reduced brain volumes in our study population.

6.3. Histological chorioamnionitis and neonatal infections in association with auditory impairment

6.3.1 BAEP

Several perinatal infections, including sepsis (Marlow et al., 2000; Meyer et al., 1999; Singh et al., 1998; Stoll et al., 2004) and NEC (Stoll et al., 2004), have been associated

with hearing deficits. Consistent with earlier studies, we found a significant association between combined CLD and/or NEC and septicaemia and prolongation of BAEP responses. Whether chorioamnionitis is a risk factor for hearing impairment has, on the other hand, not been sufficiently explored. Theoretically chorioamnionitis could have deleterious effects on the brain stem leading to hearing deficits in VLBW/VLGA infants. To our knowledge, only two study groups have previously investigated the possible association between chorioamnionitis and hearing with inconsistent results (Fung et al., 2003; Suppiej et al., 2009). Fung et al. (Fung et al., 2003) found that chorioamnionitis did not affect the incidence of hearing impairment in premature infants born before 28 weeks of gestation, and Suppiej et al. (Suppiej et al., 2009) found an association between histological chorioamnionitis and incidence of hearing loss in VLGA infants. In univariate analyses in our study population, fetal chorioamnionitis associated with prolongation of III-V inter peak interval. This association disappeared when adjusted for confounding factors, but it is still plausible that chorioamnionitis may have a deleterious effect on auditory function. The association may merely be concealed by the strong association between gestational age and chorioamnionitis. The hypothesis that placental inflammation could affect the integrity and maturation of the auditory pathway seen as changes in BAEP responses has not been previously tested.

6.3.2 DTI of the inferior colliculus

Brain white matter maturation and myelination can be evaluated using DTI, as a high FA value reflects mature, densely packed, and myelinated white matter (Beaulieu et al., 2002). The inferior colliculus has been shown to have reduced FA values in patients with sensorineural hearing impairment compared with normal hearing controls (Chang et al., 2004; Lin et al., 2008; Wu et al., 2009). Our hypothesis that histological chorioamnionitis and neonatal infections have deleterious effects on the central auditory pathway seen as changes in DTI values of the inferior colliculus was not supported by our findings, as we found that histological chorioamnionitis and neonatal infections did not to associate with DTI values of the inferior colliculus in VLBW/VLGA infants. In addition, none of our confounding factors, including gestational age and z score of birth weight, were associated with the DTI values of the inferior colliculus. To our knowledge, the association between histological chorioamnionitis and neonatal infections and DTI values of the inferior colliculus has not been previously tested. Even though the inferior colliculus is especially vulnerable for injury in the developing brain (Folkert et al., 2008) and has been suggested to be the most sensitive area in the auditory pathway to neuronal damage (Chang et al., 2004), it seems to be fairly unaffected by other risk factors, such as low gestational age and low birth weight z score, associated with altered DTI values. This may be explained by the early myelinisation of the statoacusticus system (Rorke-Adams et al., 2007), which makes it less vulnerable for the developmental problems caused by preterm delivery.

We found in our study that fetal chorioamnionitis and neonatal infections were associated with delayed BAEP latencies but were not associated with the DTI of the inferior colliculus. In addition, known risk factors of hearing impairment, low gestational age and birth weight, were also associated with BAEP results but not with DTI values. It could be postulated that if the DTI method was as sensitive as BAEP in detecting dysfunction of the brain stem auditory circuit, these risk factors would have also been associated with DTI values of the inferior colliculus. On the other hand, DTI values were correlated with BAEP responses, suggesting that, at least to some degree, DTI findings reflect the integrity of the auditory pathway in preterm infants.

6.4. IL-6 polymorphism in association with histological chorioamnionitis

The association between IL-6 promoter polymorphism and histological chorioamnionitis has not been widely studied. Only one previous study has shown a trend between the IL-6 -174 G allele and histological chorioamnionitis (Speer et al., 2006), and IL-6 -572 genotypes have not been studied in relation to chorioamnionitis at all. However, the role of cytokines, including IL-6, in chorioamnionitis seems to be indisputable, as high levels of cord blood pro-inflammatory cytokines have been connected to histological chorioamnionitis in several studies (Dollner et al., 2002; Kashlan et al., 2000; Kaukola et al., 2006; Shalak et al., 2002; Tasci et al., 2006; Yanowitz et al., 2002; Yoon et al., 2000). As IL-6 promoter polymorphisms at positions -174 and -572 have been shown to affect IL-6 response, it is plausible that these polymorphisms also associate with the incidence of histological chorioamnionitis. We evaluated the association between IL-6 -174 and -572 polymorphisms and the incidence of histological chorioamnionitis in VLBW/VLGA infants and found that both IL-6 -176 GG and -572 GC genotypes were associated with higher incidence of histological chorioamnionitis. Our study supports the finding made by Speer et al. (Speer et al., 2006) associating the IL-6 -174 GG genotype with a slightly higher incidence of histological chorioamnionitis. Even though the literature is not consistent with which IL-6 -174 genotype is related to higher IL-6 synthesis response and inflammatory response, these findings suggest it to be the GG genotype. In addition, the finding that the IL-6 -572 GC genotype associated with histological chorioamnionitis is consistent with the findings associating the C allele with increased IL-6 synthesis and thus with stronger inflammatory response.

6.5. IL-6 polymorphism in association with neonatal infections

Earlier studies have been inconsistent in regard to the association between IL-6 -174 polymorphism and septicaemia. The IL-6 -174 CC genotype has been associated with increased incidence of sepsis in pediatric intensive care patients (Michalek et al., 2007), with increased incidence of late blood stream infections in African American mechanically ventilated VLBW infants (Baier et al., 2006), and with septic shock in adults

(Tischendorf et al., 2007). On the other hand, in two other studies, the -174 GG genotype has been associated with increased incidence of septicaemia in preterm infants (Ahrens et al., 2004; Harding et al., 2003), and a number of studies have found no association between IL-6 -174 genotypes and septicaemia in preterm infants (Göpel et al., 2006; Schlüter et al., 2002). In our study, we found the IL-6-174 CC genotype to correlate with the incidence of septicaemia. This discrepancy might be explained by haplotypes, rather than genotypes, influencing IL-6 transcription, as has been suggested earlier (Terry et al., 2000). Racial differences have also been suggested to play a role in the regulation of IL-6 synthesis (Baier et al., 2006), but this is not likely in this situation, as the main population were Caucasians in most of these studies. It has also been suggested that IL-6 promoter polymorphism might be associated with organism-specific blood stream infections, as Ahrens et al. (Ahrens et al., 2004) found that the IL-6 -174 GG genotype was especially related to an increase in Gram-positive infections. However, our results do not support this finding, since in our study, from 11 infants with septicaemia, 7 had the IL-6 -174 CC genotype, and they all had Gram-positive infections.

Earlier studies have associated IL-6-572 C with a higher incidence of sepsis in pediatric intensive care unit patients (Michalek et al., 2007) and adult trauma patients (Gu et al., 2008). In our study population, we found no association betweeb IL-6 -572 genotypes to neonatal infections in univariate analyses. We were not able to perform multivariate analyses, as none of the infants with the -572 GC genotype had septicaemia, CLD, or NEC.

It is of interest that different genotypes of IL-6 -174 seemed to be predisposing to chorioamnionitis and septicaemia. However, where histological chorioamnionitis reflects the individual's inflammatory response to infectious stimuli, septicaemia is a consequence of inadequate immune system activation. It is plausible that a strong inflammatory response may indeed be protective against severe infections such as septicaemia i.e. the IL-6-174 GG genotype associates with a stronger IL-6 response resulting in better protection against serious infections.

6.6. IL-6 polymorphism in association with brain lesions

IL-6 has been shown to have multiple significant functions in the central nervous system development (Koch et al., 2007; Nakanishi et al., 2007; Taga et al., 2005), normal functioning (Braida et al., 2004), and protection from injury (Loddick et al., 1998; Matsuda et al., 1996; Penkowa et al., 2000; Winter et al., 2004) as well as being shown to be deleterious in overexposure (Campbell et al., 1993; Vallières et al., 2002). Several studies have suggested IL-6 to have a role in the process causing brain lesions in preterm infants. High levels of IL-6 in cord blood, neonatal blood, amniotic fluid, and CSF have all been associated with the development of brain pathologies, including IVH

and WMI (Ellisonn et al., 2005; Heep et al., 2003; Hitti et al., 2001; Nelson et al., 2003; Tauscher et al., 2003; Yoon et al., 1997). In addition, IL-6 -174 and -572 genotypes have been connected with deviant neurological development, brain pathologies, and mental retardation in PVL patients in preterm infants (Harding et al., 2005; Harding et al., 2004; Resch et al., 2009), suggesting that the genetic regulation of IL-6 response plays a role in the brain development of preterm infants.

In our study population, however, we did not find any association between IL-6 -174 and -572 genotypes and brain injuries. The same notion has also made by Göpel et al. (Göpel et al., 2006), as they reported no association between IL-6-174 polymorphisms and brain pathology in VLBW infants. In addition, even though IL-6 -572 genotypes have been connected with the impaired cognitive development of preterm infants (Harding et al., 2005), the same study showed no association between genotypes and brain pathology. It is possible that our rather small sample size prevented us from finding the association between genotypes and brain lesions, but as the association has been made by only one study group (Harding et al., 2004), it may be that the effects of IL-6 and the impact of IL-6 synthesis regulation are more subtle and are not shown in overt brain pathology.

6.7. IL-6 polymorphism in association with regional brain volumes

Despite not finding any association between IL-6 promoter polymorphisms and brain lesions, we did find an association between IL-6 -174 CC and -572 GG genotypes and a smaller combined volume of basal ganglia and thalami. The same genotypes were associated with a trend for smaller volumes also in the other brain regions, compared with other genotypes. IL-6 has been suggested to interfere with neurogenesis (Vallières et al., 2002) and to cause neuronal damage, reactive astrocytosis, and proliferative angiopathy (Campbell et al., 1993). The basal ganglia and thalamus are particularly vulnerable to e.g. hypoxic-ischemic insults during development (Cowan et al., 2003), so it is plausible that this area is also vulnerable to the effects of IL-6, and hence the association is only seen in the deep gray matter area. On the other hand, it may be that the association seen here is not caused by the deleterious effects of excess IL-6 exposure, but is in fact a consequence of insufficient IL-6 production. As IL-6 has been shown to protect against brain injury (Matsuda et al., 1996; Loddick et al., 1998; Winter et al., 2004), stronger IL-6 response could hence lead to better protection against the consequences of deleterious insults. For example, raised parenchymal IL-6 levels have been found to correlate with improved outcome after traumatic brain injury (Winter et al., 2004), and higher levels of IL-6 have been reported to significantly reduce ischemic brain damage after permanent focal cerebral ischemia in rat (Loddick et al., 1998) and prevented ischemia-induced learning disability and neuronal and synaptic loss in gerbils (Matsuda et al., 1996). The beneficial role of higher IL-6 production in brain volume development is in accordance with our

results, as the IL-6 -174 CC and -572 GG genotypes associated with smaller deep gray matter volume seem to be those associated with lower IL-6 synthesis response.

The IL-6 -174 CC genotype associated with smaller volumes of deep gray matter correlated with the incidence of septicaemia in our study (study II). It can be suggested that the severe infection could be a factor disturbing brain development and leading to smaller brain volumes (Shah et al., 2002). However, the associations between IL-6 -174 genotypes and the combined volume of basal ganglia and thalami did not explain the septicaemia in our study population.

6.8. Future research

The relatively small number of study infants may limit the power of our study to detect weak associations, and it may also lead to incidental findings. In addition, the number of rare complications, such as septicaemia, NEC and CLD, was low, and therefore limits the potential to draw clinical conclusions from our findings. In studies II-IV, the genetic samples and brain imaging findings were not available from the demised patients leading to a selection bias as only survivors were investigated. Another limitation, and a potential source of bias, is the fairly large number of infants excluded because of missing placental samples. Placental samples were obtained less frequently when the infant had low Apgar scores or died during the first day of life suggesting that the new policy of placental collection was not yet uniformly applied in emergency situations by the clinical staff. A potential limitation to our study is also the upgrading of the MRI equipment during the data collection period. This might affect the image contrast, and thus the ability to differentiate adjacent anatomic structures. To avoid this, we selected easily definable anatomic landmarks, which are not affected by contrast changes in MRI. In study IV, the most severely abnormal BAEP responses, i.e. the absent latencies, could not be used in the statistical analyses of latencies, thus possibly undermining the associations between chorioamnionitis and neonatal infections and BAEP responses.

Although we have intriguing preliminary findings concerning IL-6 promoter polymorphisms, it has to be kept in mind that we only analyzed two single nucleotide polymorphisms from one cytokine, when several other factors obviously also play a role in determining the effects of inflammatory situations. The other factors should be studied with larger sample size by using the modern methods of human genome research. Future studies should also be designed to include samples from non-survivors.

As our study groups were small, these results should be repeated in larger samples and in other populations before implementing the results in the clinical guidelines. In addition, a long term follow up is needed to determine the clinical significance of our findings.

CONCLUSIONS

Placental inflammation has been thought to be a risk factor for the central nervous system of preterm infants. This has also, to some degree, guided the clinical management of preterm delivery as clinical diagnosis of placental inflammation may trigger a decision to induce a preterm delivery as a precaution to protect the infant's brain (Canavan et al., 2004). Our findings give reason for reconsideration. We found no evidence that placental inflammation would pose a direct risk for brain pathology in VLBW/VLGA infants, as histological chorioamnionitis was not significantly associated with brain lesions, smaller brain volumes, or with the integrity of auditory pathway in our patient population. A recent study by Andrews et al. (Andrews et al., 2008) also suggests that chorioamnionitis is not harmful, but might even be beneficial for very preterm infants. They found that in utero exposure to acute inflammation was not associated with CP, low IQ or neurodevelopmental delay, and they even reported a trend indicating higher IQ in the presence on funisitis (Andrews et al., 2008). In addition, there is evidence that at 25 to 29 weeks of gestation, histological chorioamnionitis associates with higher survival rates in preterm infants, and that neonatal survivors have a higher incidence of fetal response to chorioamnionitis (Lahra et al., 2004). The mechanism behind this might be lung maturation induced by chorioamnionitis, as in animal models, chorioamnionitis has been shown to result in clinically significant lung maturation (Boris et al., 2009). IL-6 is one of the cytokines suggested to mediate the deleterious effects of chorioamnionitis to the infant's brain. The role of IL-6 has been thought to be merely deleterious in the process of chorioamnionitis and brain injury, but our findings give reason to draw contradictory conclusions. Our findings suggest that IL-6 promoter polymorphisms, which act functionally in the regulation of IL-6 expression, have a role in protecting against serious infections and also protecting the brain development of VLBW/VLGA infants. The genotypes associated with higher incidence of chorioamnionitis, and which thus appeared to be associated with higher inflammatory response, were associated with lower incidence of septicaemia. The genotypes associated with small volumes of deep gray matter were, quite unexpectedly, genotypes that seemed to associate with low inflammatory response. This finding gives reason to speculate that inflammatory cytokines can also have a beneficial effect on the central nervous system, and that strong inflammatory response may even be protective not only against serious infections, but also for brain development in VLBW/VLGA infants. This quite unexpected finding has also been supported by Kaukola et al. (Kaukola et al., 2006) who found that in preterm infants with histological chorioamnionitis, infants with high IL-6 concentrations had a more favourable neurologic outcome than the infants with lower IL-6 concentrations.

In conclusion, our findings suggest that histological chorioamnionitis, in itself, is not a major risk factor for the brain development of VLBW/VLGA infants, or that the risk

caused by inflammation does not outweight the risks attributed to other underlying pathologies behind preterm delivery. In addition, our findings give reason to propose a beneficial role for inflammation and IL-6 in defence against serious infections and in the brain development of VLBW/VLGA infants.

ACKNOWLEDGEMENTS

First of all, I want to express my gratitude to my supervisors docent Liisa Lehtonen and docent Leena Haataja. I feel that your positive feedback, encouragement, and support really made this journey worth while. I was never left alone with my problems, as there never seemed to be a bad time to ask help and guidance. You made me feel proud of what I was doing, even though I was often contemplating the meaning of my work. It might be my thesis, but you made me the researcher I am today as I'm finally at the end of this chapter of my life. A thank you also goes to docent Helena Lapinleimu, who was supposed to be one of my supervisors, who I always thought of as my supervisor, and who always treated me as she was my supervisor. You had a major input in this work, and I'm deeply grateful for that.

I was privileged to have Professor Seppo Heinonen, and docent Martin Renlund as the official reviewers of this thesis. Their wise comments and constructive criticism helped me improve the quality of my work, and I am grateful for that.

I want to thank all of my co-writers for their contribution to this thesis and the original publications. I also want to thank you for the collaboration and encouragement during this long process. Special thanks go to Harry Kujari for his work with the placental samples and also for his encouragement and friendship. Riitta Parkkola, you also put an invaluable effort for this thesis. Without your expertise and knowledge and patience this work would have never been possible. I also wish to thank the rest of the PIPARI study group for your input in my work, and also for your friendship and encouragement. You have created warm and encouraging environment for research work, and as much as I value the expertise you have all provided for this work, I am also deeply grateful for all the dear friends I have made. I am in gratitude for Kukka Aimonen and Kirsi Huoponen for the work with the DNA samples. And Jaakko Matomäki, this work would never have been finished without your work wiht the statistics. I don't know how you have the patience to explain these things to me over and over again.

I also wish to thank all of the families participating in PIPARI study. You give us invaluable information, and make this study possible.

All my co-workers in the research unit are warmly acknowledged by the support and friendship you have given me through these years. All the coffee breaks and conversations have made even the hard days tolerable. You have provided invaluable therapy to get me through the bad days, and what a joy to have you to share the achievements of the research life whit!

I also want to show my appreciation to all of my dear friends outside the research world. Thank you for sharing the ups and downs of research life and life in general. Thank you for making me forget my responsibilities once in a while and thank you for enjoying the life with me!

I am deeply grateful for my family, and especially for my parents Maikki and Markku. Thank you for providing me all the loving and support during my life. You have always made me feel proud of my self, and you have made me believe that nothing is impossible. You have made me the person I am today, a person who is not afraid to take on challenges, a person who believes in her self, and a person who is proud of what she has achieved and to be who she is.

And Joonas, my love and my best friend, you deserve the greatest gratitude. Thank you for your love and support. Thank you for sharing the ups and downs with me. Thank you for pushing me forward when I was in need for it, and thank you for making me slow down when I was going on to fast. Thank you for taking care of our "babies" when I was too busy working, and thank you for not complaining about it. Thank you for being everything that you are to me, thank you for making the life worth living.

This study was carried out in the Department of Pediatrics, Turku University Hospital, Turku, Finland, during the years 2004-2009. The research office was provided by the Turku University Hospital foundation. This study was supported by grants from the Academy of Finland, the South-Western Finnish Foundation of Neonatal Research, the Foundation for Pediatric Research, the Lea and Arvo Ylppö Foundation, the Päivikki and Sakari Sohlberg Foundation, and the Emil Aaltonen Foundation.

REFERENCES

Aaltonen R, Heikkinen T, Hakala K, Laine K, Alanen A. Transfer of proinflammatory cytokines across term placenta. Obstet.Gynecol. 2005 Oct;106(4):802-807.

Abernethy LJ, Cooke RW, Foulder-Hughes L. Caudate and hippocampal volumes, intelligence, and motor impairment in 7-year-old children who were born preterm. Pediatr.Res. 2004 May;55(5):884-893.

Abernethy LJ, Palaniappan M, Cooke RW. Quantitative magnetic resonance imaging of the brain in survivors of very low birth weight. Arch.Dis.Child. 2002 Oct;87(4):279-283.

Ahrens P, Kattner E, Kohler B, Hartel C, Seidenberg J, Segerer H, et al. Mutations of genes involved in the innate immune system as predictors of sepsis in very low birth weight infants. Pediatr. Res. 2004 Apr;55(4):652-656.

Alexander JM, Gilstrap LC, Cox SM, McIntire DM, Leveno KJ. Clinical chorioamnionitis and the prognosis for very low birth weight infants. Obstet.Gynecol. 1998 May;91(5 Pt 1):725-729.

Allin M, Matsumoto H, Santhouse AM, Nosarti C, AlAsady MH, Stewart AL, et al. Cognitive and motor function and the size of the cerebellum in adolescents born very pre-term. Brain 2001 Jan;124(Pt 1):60-66.

An H, Nishimaki S, Ohyama M, Haruki A, Naruto T, Kobayashi N, et al. Interleukin-6, interleukin-8, and soluble tumor necrosis factor receptor-I in the cord blood as predictors of chronic lung disease in premature infants. Am.J.Obstet. Gynecol. 2004 Nov;191(5):1649-1654.

Andrews WW, Cliver SP, Biasini F, Peralta-Carcelen AM, Rector R, Alriksson-Schmidt AI, et al. Early preterm birth: association between in utero exposure to acute inflammation and severe neurodevelopmental disability at 6 years of age. Am.J.Obstet.Gynecol. 2008 Apr;198(4):466.e1-466.e11.

Andrews WW, Hauth JC, Goldenberg RL. Infection and preterm birth. Am J Perinatol. 2000;17(7):357-65

Anjari M, Srinivasan L, Allsop JM, Hajnal JV, Rutherford MA, Edwards AD, et al. Diffusion tensor imaging with tract-based spatial statistics reveals local white matter abnormalities in preterm infants. Neuroimage 2007 Apr 15;35(3):1021-1027.

Annells MF, Hart PH, Mullighan GC, Heatley SL, Robinson JS, Bardy P, et al. Interleukins-1, -4, -6, -10, tumor necrosis factor, transforming growth factor-β, FAS, and mannose-binding protein C gene polymorphisms in Australian women: Risk of preterm birth. Am J Obstet Gynecol. 2004 Dec;191(6):2056-2067.

Arpino C, D'Argenzio L, Ticconi C, Di Paolo A, Stellin V, Lopez L, et al. Brain damage in preterm infants: etiological pathways. Ann.Ist. Super.Sanita 2005;41(2):229-237.

Baier RJ, Loggins J, Yanamandra K. IL-10, IL-6 and CD14 polymorphisms and sepsis outcome in ventilated very low birth weight infants. BMC Med. 2006 Apr 12;4:10.

Bao X, Wong V. Brainstem auditory-evoked potential evaluation in children with meningitis. Pediatr.Neurol. 1998 Aug;19(2):109-112.

Barbi M, Binda S, Caroppo S, Ambrosetti U, Corbetta C, Sergi P. A wider role for congenital cytomegalovirus infection in sensorineural hearing loss. Pediatr.Infect.Dis.J. 2003 Jan;22(1):39-42.

Barbi M, Binda S, Caroppo S, Calvario A, Germinario C, Bozzi A, et al. Multicity Italian study of congenital cytomegalovirus infection. Pediatr.Infect.Dis.J. 2006 Feb;25(2):156-159.

Barkovich AJ. Pediatric neuroimaging. Raven Press, New York, 1995, pp 120-128

Bartha AI, Foster-Barber A, Miller SP, Vigneron DB, Glidden DV, Barkovich AJ, et al. Neonatal encephalopathy: association of cytokines with MR spectroscopy and outcome. Pediatr.Res. 2004 Dec;56(6):960-966.

Beauchamp MH, Thompson DK, Howard K, Doyle LW, Egan GF, Inder TE, et al. Preterm infant hippocampal volumes correlate with later working memory deficits. Brain 2008 Nov;131(Pt 11):2986-2994.

Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed. 2002 Nov-Dec;15(7-8):435-455.

Bejar R, Wozniak P, Allard M, Benirschke K, Vaucher Y, Coen R, et al. Antenatal origin of neurologic damage in newborn infants. I. Preterm infants. Am.J.Obstet.Gynecol. 1988 Aug;159(2):357-363.

Berkovic MC, Jokic M, Marout J, Radosevic S, Zjacic-Rotkvic V, Kapitanovic S. IL-6-174 C/

G polymorphism in the gastroenteropancreatic neuroendocrine tumors (GEP-NETs). Exp.Mol. Pathol. 2007 Dec;83(3):474-479.

Berman JI, Mukherjee P, Partridge SC, Miller SP, Ferriero DM, Barkovich AJ, et al. Quantitative diffusion tensor MRI fiber tractography of sensorimotor white matter development in premature infants. Neuroimage 2005 Oct 1;27(4):862-871.

Blume HK, Li CI, Loch CM, Koepsell TD. Intrapartum fever and chorioamnionitis as risks for encephalopathy in term newborns: a case-control study. Dev.Med.Child Neurol. 2008 Jan;50(1):19-24.

Boardman JP, Counsell SJ, Rueckert D, Hajnal JV, Bhatia KK, Srinivasan L, et al. Early growth in brain volume is preserved in the majority of preterm infants. Ann.Neurol. 2007 Aug;62(2):185-192.

Boardman JP, Counsell SJ, Rueckert D, Kapellou O, Bhatia KK, Aljabar P, et al. Abnormal deep grey matter development following preterm birth detected using deformation-based morphometry. Neuroimage 2006 Aug 1;32(1):70-78.

Kramer BW, Kallapur S, Newnham J, Jobe AH. Prenatal inflammation and lung development. Semin Fetal Neonatal Med. 2009 Feb;14(1):2-7.

Braida D, Sacerdote P, Panerai AE, Bianchi M, Aloisi AM, Iosue S, et al. Cognitive function in young and adult IL (interleukin)-6 deficient mice. Behav.Brain Res. 2004 Aug 31;153(2):423-429.

Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, Rumley A, et al. Interleukin-6 gene -174g>c and -572g>c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler.Thromb.Vasc.Biol. 2001 Sep;21(9):1458-1463.

Campbell IL, Abraham CR, Masliah E, Kemper P, Inglis JD, Oldstone MB, et al. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. Proc.Natl.Acad. Sci.U.S.A. 1993 Nov 1;90(21):10061-10065.

Canavan TP, Simhan HN, Caritis S. An evidence-based approach to the evaluation and treatment of premature rupture of membranes: Part II. Obstet.Gynecol.Surv. 2004 Sep;59(9):678-689.

Castellucci L, Menezes E, Oliveira J, Magalhaes A, Guimaraes LH, Lessa M, et al. IL6 -174 G/C promoter polymorphism influences susceptibility to mucosal but not localized cutaneous leishmaniasis in Brazil. J.Infect.Dis. 2006 Aug 15;194(4):519-527.

Chang Y, Lee SH, Lee YJ, Hwang MJ, Bae SJ, Kim MN, et al. Auditory neural pathway evaluation on sensorineural hearing loss using diffusion tensor imaging. Neuroreport 2004 Aug 6;15(11):1699-1703.

Fagan SJ, Byrd SE. Normal anatomy and development of the brain. In: Cohen MD and Edwards MK. Magnetic resonance imaging of children. B.C. Decker, Inc. 1990, Philadelphia, Pennsylvania, USA. pp. 85-99

Costantine MM, How HY, Coppage K, Maxwell RA, Sibai BM. Does peripartum infection increase the incidence of cerebral palsy in extremely low birthweight infants? Am J Obstet Gynecol. 2007 May;196(5):e6-8.

Counsell SJ, Allsop JM, Harrison MC, Larkman DJ, Kennea NL, Kapellou O, et al. Diffusion-weighted imaging of the brain in preterm infants with focal and diffuse white matter abnormality. Pediatrics 2003 Jul;112(1 Pt 1):1-7.

Cowan F, Rutherford M, Groenendaal F, Eken P, Mercuri E, Bydder GM, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. Lancet 2003 Mar 1;361(9359):736-742.

Curry AE, Vogel I, Drews C, Schendel D, Skogstrand K, Flanders WD, et al. Mid-pregnancy maternal plasma levels of interleukin 2, 6, and 12, tumor necrosis factor-alpha, interferon-gamma, and granulocyte-macrophage colony-stimulating factor and spontaneous preterm delivery. Acta Obstet. Gynecol.Scand. 2007;86(9):1103-1110.

Das VK. Aetiology of bilateral sensorineural hearing impairment in children: a 10 year study. Arch.Dis.Child. 1996 Jan;74(1):8-12.

DiSalvo D. The correlation between placental pathology and intraventricular hemorrhage in the preterm infant. The Developmental Epidemiology Network Investigators. Pediatr. Res. 1998 Jan;43(1):15-19.

D'Mello J. High risk register--an economical tool for early identification of hearing loss. Indian J.Pediatr. 1995 Nov-Dec;62(6):731-735.

Dollner H, Vatten L, Halgunset J, Rahimipoor S, Austgulen R. Histologic chorioamnionitis and umbilical serum levels of pro-inflammatory cytokines and cytokine inhibitors. BJOG 2002 May;109(5):534-539.

du Plessis AJ, Volpe JJ. Perinatal brain injury in the preterm and term newborn. Curr.Opin.Neurol. 2002 Apr;15(2):151-157.

Dudink J, Kerr JL, Paterson K, Counsell SJ. Connecting the developing preterm brain. Early Hum.Dev. 2008 Dec;84(12):777-782.

Dudink J, Lequin M, van Pul C, Buijs J, Conneman N, van Goudoever J, et al. Fractional anisotropy in white matter tracts of very-low-birth-weight infants. Pediatr.Radiol. 2007 Dec;37(12):1216-1223.

Ellison VJ, Mocatta TJ, Winterbourn CC, Darlow BA, Volpe JJ, Inder TE. The relationship of CSF and plasma cytokine levels to cerebral white matter injury in the premature newborn. Pediatr. Res. 2005 Feb;57(2):282-286.

Emonts M, Veenhoven RH, Wiertsema SP, Houwing-Duistermaat JJ, Walraven V, de Groot R, et al. Genetic polymorphisms in immunoresponse genes TNFA, IL6, IL10, and TLR4 are associated with recurrent acute otitis media. Pediatrics 2007 Oct;120(4):814-823.

Fearon P, O'Connell P, Frangou S, Aquino P, Nosarti C, Allin M, et al. Brain volumes in adult survivors of very low birth weight: a sibling-controlled study. Pediatrics 2004 Aug;114(2):367-371.

Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J.Clin.Invest. 1998 Oct 1;102(7):1369-1376.

Folkert RD, Kinney HC. Disorders of the perinatal period. In: Love S, Louis DN, Ellison DW. Greenfield's Neuropathology. Eight edition. London: Hodden Arnold; 2008. pp. 241-315.

Forbes KP, Pipe JG, Bird CR. Changes in brain water diffusion during the 1st year of life. Radiology 2002 Feb;222(2):405-409.

Fowler KB, Boppana SB. Congenital cytomegalovirus (CMV) infection and hearing deficit. J.Clin.Virol. 2006 Feb;35(2):226-231.

Fung G, Bawden K, Chow P, Yu V. Chorioamnionitis and outcome in extremely preterm infants. Ann. Acad.Med.Singapore 2003 May;32(3):305-310.

Gargano JW, Holzman C, Senagore P, Thorsen P, Skogstrand K, Hougaard DM, et al. Mid-pregnancy circulating cytokine levels, histologic chorioamnionitis and spontaneous preterm birth. J.Reprod.Immunol. 2008 Oct;79(1):100-110.

Gilmore JH, Zhai G, Wilber K, Smith JK, Lin W, Gerig G. 3 Tesla magnetic resonance imaging of the brain in newborns. Psychiatry Res. 2004 Nov 15;132(1):81-85.

Gimenez M, Junque C, Narberhaus A, Bargallo N, Botet F, Mercader JM. White matter volume and concentration reductions in adolescents with history of very preterm birth: a voxel-based morphometry study. Neuroimage 2006 Oct 1;32(4):1485-1498.

Goepfert AR, Andrews WW, Carlo W, Ramsey PS, Cliver SP, Goldenberg RL, et al. Umbilical cord plasma interleukin-6 concentrations in preterm infants and risk of neonatal morbidity. Am.J.Obstet.Gynecol. 2004 Oct;191(4):1375-1381.

Goldenberg RL. The management of preterm labor. Obstet.Gynecol. 2002 Nov;100(5 Pt 1):1020-1037.

Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008 Jan 5;371(9606):75-84.

Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000 May 18;342(20):1500-7

Gopel W, Hartel C, Ahrens P, Konig I, Kattner E, Kuhls E, et al. Interleukin-6-174-genotype, sepsis and cerebral injury in very low birth weight infants. Genes Immun. 2006 Jan;7(1):65-68.

Gray PH, Jones P, O'Callaghan MJ. Maternal antecedents for cerebral palsy in extremely preterm babies: a case-control study. Dev.Med. Child Neurol. 2001 Sep;43(9):580-585.

Grether JK, Nelson KB. Maternal infection and cerebral palsy in infants of normal birth weight. JAMA 1997 Jul 16;278(3):207-211.

Grether JK, Nelson KB, Walsh E, Willoughby RE, Redline RW. Intrauterine exposure to infection and risk of cerebral palsy in very preterm infants. Arch.Pediatr.Adolesc.Med. 2003 Jan;157(1):26-32.

Gu W, Du DY, Huang J, Zhang LY, Liu Q, Zhu PF, et al. Identification of interleukin-6 promoter polymorphisms in the Chinese Han population and their functional significance. Crit.Care Med. 2008 May;36(5):1437-1443.

Hansen-Pupp I, Hallin AL, Hellstrom-Westas L, Cilio C, Berg AC, Stjernqvist K, et al. Inflammation at birth is associated with subnormal development in very preterm infants. Pediatr.Res. 2008 Aug;64(2):183-188.

Harding D, Brull D, Humphries SE, Whitelaw A, Montgomery H, Marlow N. Variation in the interleukin-6 gene is associated with impaired cognitive development in children born prematurely: a preliminary study. Pediatr.Res. 2005 Jul;58(1):117-120.

Harding D, Dhamrait S, Millar A, Humphries S, Marlow N, Whitelaw A, et al. Is interleukin-6 -174 genotype associated with the development of septicemia in preterm infants? Pediatrics 2003 Oct;112(4):800-803.

Harding DR, Dhamrait S, Whitelaw A, Humphries SE, Marlow N, Montgomery HE. Does interleukin-6 genotype influence cerebral injury or developmental progress after preterm birth? Pediatrics 2004 Oct;114(4):941-947.

Hatzidaki E, Giahnakis E, Maraka S, Korakaki E, Manoura A, Saitakis E, et al. Risk factors for periventricular leukomalacia. Acta Obstet. Gynecol.Scand. 2009;88(1):110-115.

Heep A, Behrendt D, Nitsch P, Fimmers R, Bartmann P, Dembinski J. Increased serum levels of interleukin 6 are associated with severe intraventricular haemorrhage in extremely premature infants. Arch Dis Child Fetal Neonatal Ed. 2003 Nov;88(6):F501-4.

Heesen M, Obertacke U, Schade FU, Bloemeke B, Majetschak M. The interleukin-6 G(-174)C polymorphism and the ex vivo interleukin-6 response to endotoxin in severely injured blunt trauma patients. Eur.Cytokine Netw. 2002 Jan-Mar;13(1):72-77.

Hitti J, Tarczy-Hornoch P, Murphy J, Hillier SL, Aura J, Eschenbach DA. Amniotic fluid infection, cytokines, and adverse outcome among infants at 34 weeks' gestation or less. Obstet.Gynecol. 2001 Dec;98(6):1080-1088.

Holcroft CJ, Blakemore KJ, Allen M, Graham EM. Association of prematurity and neonatal infection with neurologic morbidity in very low birth weight infants. Obstet.Gynecol. 2003 Jun;101(6):1249-1253.

Hulkkonen J, Pertovaara M, Antonen J, Pasternack A, Hurme M. Elevated interleukin-6 plasma levels are regulated by the promoter region polymorphism of the IL6 gene in primary Sjogren's syndrome and correlate with the clinical manifestations of the disease. Rheumatology (Oxford) 2001 Jun;40(6):656-661.

Huppi PS, Maier SE, Peled S, Zientara GP, Barnes PD, Jolesz FA, et al. Microstructural development of human newborn cerebral white matter assessed in vivo by diffusion tensor magnetic resonance imaging. Pediatr.Res. 1998 Oct;44(4):584-590.

Huppi PS, Murphy B, Maier SE, Zientara GP, Inder TE, Barnes PD, et al. Microstructural brain development after perinatal cerebral white matter injury assessed by diffusion tensor magnetic resonance imaging. Pediatrics 2001 Mar;107(3):455-460.

Inder TE, Huppi PS, Warfield S, Kikinis R, Zientara GP, Barnes PD, et al. Periventricular white matter injury in the premature infant is followed by reduced cerebral cortical gray matter volume at term. Ann.Neurol. 1999 Nov;46(5):755-760.

Inder TE, Volpe JJ. Mechanisms of perinatal brain injury. Semin.Neonatol. 2000 Feb;5(1):3-16.

Inder TE, Warfield SK, Wang H, Huppi PS, Volpe JJ. Abnormal cerebral structure is present at term in premature infants. Pediatrics 2005 Feb;115(2):286-294.

Isaacs EB, Lucas A, Chong WK, Wood SJ, Johnson CL, Marshall C, et al. Hippocampal volume and everyday memory in children of very low birth weight. Pediatr.Res. 2000 Jun;47(6):713-720.

Iwasaki S, Yamashita M, Maeda M, Misawa K, Mineta H. Audiological outcome of infants with congenital cytomegalovirus infection in a prospective study. Audiol.Neurootol. 2007;12(1):31-36.

Jacobsson B, Hagberg G, Hagberg B, Ladfors L, Niklasson A, Hagberg H. Cerebral palsy in preterm infants: a population-based case-control study of antenatal and intrapartal risk factors. Acta Paediatr. 2002;91(8):946-951.

Jamie WE, Edwards RK, Ferguson RJ, Duff P. The interleukin-6--174 single nucleotide polymorphism: cervical protein production and the risk of preterm delivery. Am J Obstet Gynecol. 2005 Apr;192(4):1023-1027.

Janeway CA, Travers P, Walport M, Shlomchik MJ. Immunobiology 6th ed. Garland Science Publishing. 2005: pp. 823

Jang Y, Kim OY, Hyun YJ, Chae JS, Koh SJ, Heo YM, et al. Interleukin-6-572C>G polymorphism-association with inflammatory variables in Korean men with coronary artery disease. Transl.Res. 2008 Mar;151(3):154-161.

Jenny NS, Tracy RP, Ogg MS, Luong le A, Kuller LH, Arnold AM, et al. In the elderly, interleukin-6 plasma levels and the -174G>C polymorphism are associated with the development of cardiovascular disease. Arterioscler.Thromb. Vasc.Biol. 2002 Dec 1;22(12):2066-2071.

Jiang ZD, Brosi DM, Wilkinson AR. Hearing impairment in preterm very low birthweight babies detected at term by brainstem auditory evoked responses. Acta Paediatr. 2001 Dec;90(12):1411-1415.

Jones KG, Brull DJ, Brown LC, Sian M, Greenhalgh RM, Humphries SE, et al. Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. Circulation 2001 May 8;103(18):2260-2265.

Kashlan F, Smulian J, Shen-Schwarz S, Anwar M, Hiatt M, Hegyi T. Umbilical vein interleukin 6 and tumor necrosis factor alpha plasma concentrations in the very preterm infant. Pediatr.Infect.Dis.J. 2000 Mar;19(3):238-243.

Kaukola T, Herva R, Perhomaa M, Paakko E, Kingsmore S, Vainionpaa L, et al. Population cohort associating chorioamnionitis, cord inflammatory cytokines and neurologic outcome in very preterm, extremely low birth weight infants. Pediatr.Res. 2006 Mar;59(3):478-483.

Kesler SR, Reiss AL, Vohr B, Watson C, Schneider KC, Katz KH, et al. Brain volume reductions within multiple cognitive systems in male preterm children at age twelve. J.Pediatr. 2008 Apr;152(4):513-20, 520.e1.

Kilpinen S, Hulkkonen J, Wang XY, Hurme M. The promoter polymorphism of the interleukin-6 gene regulates interleukin-6 production in neonates but not in adults. Eur.Cytokine Netw. 2001 Mar;12(1):62-68.

Kiszel P, Mako V, Prohaszka Z, Cervenak L. Interleukin-6 -174 promoter polymorphism does not influence IL-6 production after LPS and IL-1 beta stimulation in human umbilical cord vein endothelial cells. Cytokine 2007 Oct;40(1):17-22.

Koch M, May U, Kuhns S, Drechsler H, Adam N, Hattermann K, et al. Interleukin 27 induces differentiation of neural C6-precursor cells into astrocytes. Biochem.Biophys.Res.Commun. 2007 Dec 21;364(3):483-487.

Koh SJ, Jang Y, Hyun YJ, Park JY, Song YD, Shin KK, et al. Interleukin-6 (IL-6) -572C-->G promoter polymorphism is associated with type 2 diabetes risk in Koreans. Clin.Endocrinol.(Oxf) 2009 Feb;70(2):238-244.

Korres S, Nikolopoulos TP, Komkotou V, Balatsouras D, Kandiloros D, Constantinou D, et al. Newborn hearing screening: effectiveness, importance of high-risk factors, and characteristics of infants in the neonatal intensive care unit and well-baby nursery. Otol.Neurotol. 2005 Nov;26(6):1186-1190.

Korvenranta E, Lehtonen L, Peltola M, Häkkinen U, Andersson S, Gissler M, et al. Morbidities and Hospital Resource Use During the First 3 Years of Life Among Very Preterm Infants. Pediatrics 2009;124:128-134

Kostovic I, Jovanov-Milosevic N. The development of cerebral connections during the first 20-45 weeks' gestation. Semin.Fetal.Neonatal Med. 2006 Dec;11(6):415-422.

Kumazaki K, Nakayama M, Sumida Y, Ozono K, Mushiake S, Suehara N, et al. Placental features in preterm infants with periventricular leukomalacia. Pediatrics 2002 Apr;109(4):650-655.

Kurkinen-Raty M, Ruokonen A, Vuopala S, Koskela M, Rutanen EM, Karkkainen T, et al. Combination of cervical interleukin-6 and -8, phosphorylated insulin-like growth factor-binding protein-1 and transvaginal cervical ultrasonography in assessment of the risk of preterm birth. BJOG 2001 Aug;108(8):875-881.

Laborada G, Nesin M. Interleukin-6 and interleukin-8 are elevated in the cerebrospinal fluid of infants exposed to chorioamnionitis. Biol.Neonate 2005;88(2):136-144.

Lahra MM, Jeffery HE. A fetal response to chorioamnionitis is associated with early survival after preterm birth. Am.J.Obstet.Gynecol. 2004 Jan;190(1):147-151.

Lapointe A, Viamonte C, Morriss MC, Manolidis S. Central nervous system findings by magnetic resonance in children with profound sensorineural hearing loss. Int J Pediatr Otorhinolaryngol. 2006 May;70(5):863-868.

Leviton A, Paneth N, Reuss ML, Susser M, Allred EN, Dammann O, et al. Maternal infection, fetal inflammatory response, and brain damage in very low birth weight infants. Developmental Epidemiology Network Investigators. Pediatr. Res. 1999 Nov;46(5):566-575.

Limperopoulos C, Soul JS, Gauvreau K, Huppi PS, Warfield SK, Bassan H, et al. Late gestation cerebellar growth is rapid and impeded by premature birth. Pediatrics 2005 Mar;115(3):688-695.

Lin Y, Okumura A, Hayakawa F, Kato K, Kuno T, Watanabe K. Quantitative evaluation of thalami and basal ganglia in infants with periventricular leukomalacia. Dev.Med.Child Neurol. 2001 Jul;43(7):481-485.

Lin Y, Wang J, Wu C, Wai Y, Yu J, Ng S. Diffusion tensor imaging of the auditory pathway in sensorineural hearing loss: changes in radial

diffusivity and diffusion anisotropy. J.Magn. Reson.Imaging 2008 Sep;28(3):598-603.

Loddick SA, Turnbull AV, Rothwell NJ. Cerebral interleukin-6 is neuroprotective during permanent focal cerebral ischemia in the rat. J.Cereb.Blood Flow Metab. 1998 Feb;18(2):176-179.

Malarstig A, Wallentin L, Siegbahn A. Genetic variation in the interleukin-6 gene in relation to risk and outcomes in acute coronary syndrome. Thromb.Res. 2007;119(4):467-473.

Marcus GM, Whooley MA, Glidden DV, Pawlikowska L, Zaroff JG, Olgin JE. Interleukin-6 and atrial fibrillation in patients with coronary artery disease: data from the Heart and Soul Study. Am.Heart J. 2008 Feb;155(2):303-309.

Marlow ES, Hunt LP, Marlow N. Sensorineural hearing loss and prematurity. Arch.Dis.Child. Fetal Neonatal Ed. 2000 Mar;82(2):F141-4.

Matsuda S, Wen TC, Morita F, Otsuka H, Igase K, Yoshimura H, et al. Interleukin-6 prevents ischemia-induced learning disability and neuronal and synaptic loss in gerbils. Neurosci. Lett. 1996 Feb 2;204(1-2):109-112.

Mattar R, de Souza E, Daher S. Preterm delivery and cytokine gene polymorphisms. J Reprod Med. 2006 Apr;51(4):317-320.

Mayr J, Fasching G, Hollwarth ME. Psychosocial and psychomotoric development of very low birthweight infants with necrotizing enterocolitis. Acta Paediatr.Suppl. 1994;396:96-100.

McGraw P, Liang L, Provenzale JM. Evaluation of normal age-related changes in anisotropy during infancy and childhood as shown by diffusion tensor imaging. AJR Am.J.Roentgenol. 2002 Dec;179(6):1515-1522.

Menon R, Camargo MC, Thorsen P, Lombardi SJ, Fortunato SJ. Amniotic fluid interleukin-6 increase is an indicator of spontaneous preterm birth in white but not black Americans. Am.J.Obstet.Gynecol. 2008 Jan;198(1):77.e1-77.e7.

Mewes AU, Huppi PS, Als H, Rybicki FJ, Inder TE, McAnulty GB, et al. Regional brain development in serial magnetic resonance imaging of low-risk preterm infants. Pediatrics 2006 Jul;118(1):23-33.

Meyer C, Witte J, Hildmann A, Hennecke KH, Schunck KU, Maul K, et al. Neonatal screening for hearing disorders in infants at risk: incidence, risk factors, and follow-up. Pediatrics 1999 Oct;104(4 Pt 1):900-904.

Michalek J, Svetlikova P, Fedora M, Klimovic M, Klapacova L, Bartosova D, et al. Interleukin-6 gene variants and the risk of sepsis development in children. Hum.Immunol. 2007 Sep;68(9):756-760.

Miller SP, Vigneron DB, Henry RG, Bohland MA, Ceppi-Cozzio C, Hoffman C, et al. Serial quantitative diffusion tensor MRI of the premature brain: development in newborns with and without injury. J.Magn.Reson.Imaging 2002 Dec;16(6):621-632.

Morgan L, Cooper J, Montgomery H, Kitchen N, Humphries SE. The interleukin-6 gene -174G>C and -572G>C promoter polymorphisms are related to cerebral aneurysms. J.Neurol. Neurosurg.Psychiatry. 2006 Aug;77(8):915-917.

Morlet T, Ferber-Viart C, Putet G, Sevin F, Duclaux R. Auditory screening in high-risk pre-term and full-term neonates using transient evoked otoacoustic emissions and brainstem auditory evoked potentials. Int.J.Pediatr. Otorhinolaryngol. 1998 Sep 15;45(1):31-40.

Moura E, Mattar R, de Souza E, Torloni MR, Gonçalves-Primo A, Daher S. Inflammatory cytokine gene polymorphisms and spontaneous preterm birth. J Reprod Immunol. 2009 Apr 16. [Epub ahead of print]

Murata Y, Itakura A, Matsuzawa K, Okumura A, Wakai K, Mizutani S. Possible antenatal and perinatal related factors in development of cystic periventricular leukomalacia. Brain Dev. 2005 Jan;27(1):17-21.

National Birthregister. Parturities, deliveries and Births 2007. Statistical summary. Official statistics of Finland Health 2007. Stakes http://www.stakes.fi/EN/tilastot/statisticsbytopic/reproduction/parturients.htm

Nakanishi M, Niidome T, Matsuda S, Akaike A, Kihara T, Sugimoto H. Microglia-derived interleukin-6 and leukaemia inhibitory factor promote astrocytic differentiation of neural stem/progenitor cells. Eur.J.Neurosci. 2007 Feb;25(3):649-658.

Neil JJ, Shiran SI, McKinstry RC, Schefft GL, Snyder AZ, Almli CR, et al. Normal brain in human newborns: apparent diffusion coefficient and diffusion anisotropy measured by using diffusion tensor MR imaging. Radiology 1998 Oct;209(1):57-66.

Nelson KB, Grether JK, Dambrosia JM, Walsh E, Kohler S, Satyanarayana G, et al. Neonatal cytokines and cerebral palsy in very preterm infants. Pediatr.Res. 2003 Apr;53(4):600-607.

Nieters A, Brems S, Becker N. Cross-sectional study on cytokine polymorphisms, cytokine production after T-cell stimulation and clinical parameters in a random sample of a German population. Hum.Genet. 2001 Mar;108(3):241-248.

Noponen-Hietala N, Virtanen I, Karttunen R, Schwenke S, Jakkula E, Li H, et al. Genetic variations in IL6 associate with intervertebral disc disease characterized by sciatica. Pain 2005 Mar;114(1-2):186-194.

Nosarti C, Al-Asady MH, Frangou S, Stewart AL, Rifkin L, Murray RM. Adolescents who were born very preterm have decreased brain volumes. Brain 2002 Jul;125(Pt 7):1616-1623.

Nosarti C, Allin MP, Frangou S, Rifkin L, Murray RM. Hyperactivity in adolescents born very preterm is associated with decreased caudate volume. Biol.Psychiatry 2005 Mar 15;57(6):661-666.

Nosarti C, Giouroukou E, Healy E, Rifkin L, Walshe M, Reichenberg A, et al. Grey and white matter distribution in very preterm adolescents mediates neurodevelopmental outcome. Brain 2008 Jan;131(Pt 1):205-217.

Ogawa H, Suzutani T, Baba Y, Koyano S, Nozawa N, Ishibashi K, et al. Etiology of severe sensorineural hearing loss in children: independent impact of congenital cytomegalovirus infection and GJB2 mutations. J.Infect.Dis. 2007 Mar 15;195(6):782-788.

O'Shea TM, Klinepeter KL, Dillard RG. Prenatal events and the risk of cerebral palsy in very low birth weight infants. Am.J.Epidemiol. 1998 Feb 15;147(4):362-369.

Paananen R, Husa AK, Vuolteenaho R, Herva R, Kaukola T, Hallman M. Blood cytokines during the perinatal period in very preterm infants: relationship of inflammatory response and bronchopulmonary dysplasia. J.Pediatr. 2009 Jan;154(1):39-43.e3.

Pacora P, Chaiworapongsa T, Maymon E, Kim YM, Gomez R, Yoon BH, et al. Funisitis and chorionic vasculitis: the histological counterpart of the fetal inflammatory response syndrome. J.Matern. Fetal.Neonatal Med. 2002 Jan;11(1):18-25.

Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. Ann.Intern. Med. 1998 Jan 15;128(2):127-137.

Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J.Pediatr. 1978 Apr;92(4):529-534.

Patel JA, Nair S, Revai K, Grady J, Saeed K, Matalon R, et al. Association of proinflammatory cytokine gene polymorphisms with susceptibility to otitis media. Pediatrics 2006 Dec;118(6):2273-2279.

Patra K, Wilson-Costello D, Taylor HG, Mercuri-Minich N, Hack M. Grades I-II intraventricular hemorrhage in extremely low birth weight infants: effects on neurodevelopment. J.Pediatr. 2006 Aug;149(2):169-173.

Penkowa M, Giralt M, Carrasco J, Hadberg H, Hidalgo J. Impaired inflammatory response and increased oxidative stress and neurodegeneration after brain injury in interleukin-6-deficient mice. Glia 2000 Dec;32(3):271-285.

Peterson BS, Anderson AW, Ehrenkranz R, Staib LH, Tageldin M, Colson E, et al. Regional brain volumes and their later neurodevelopmental correlates in term and preterm infants. Pediatrics 2003 May;111(5 Pt 1):939-948.

Peterson BS, Vohr B, Staib LH, Cannistraci CJ, Dolberg A, Schneider KC, et al. Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. JAMA 2000 Oct 18;284(15):1939-1947.

Polam S, Koons A, Anwar M, Shen-Schwarz S, Hegyi T. Effect of chorioamnionitis on neurodevelopmental outcome in preterm infants. Arch.Pediatr.Adolesc.Med. 2005 Nov;159(11):1032-1035.

Potaczek DP, Undas A, Nowakowski T, Szczeklik A. Association between inflammatory markers and the interleukin-6 -174 G/C polymorphism is abolished in peripheral arterial occlusive disease. Int.Angiol. 2007 Dec;26(4):318-323.

Rademaker KJ, Uiterwaal CS, Beek FJ, van Haastert IC, Lieftink AF, Groenendaal F, et al. Neonatal cranial ultrasound versus MRI and neurodevelopmental outcome at school age in children born preterm. Arch.Dis.Child.Fetal Neonatal Ed. 2005 Nov;90(6):F489-93.

Raunio T, Nixdorf M, Knuuttila M, Karttunen R, Vainio O, Tervonen T. The extent of periodontal disease and the IL-6 -174 genotype as determinants of serum IL-6 level. J.Clin. Periodontol. 2007 Dec;34(12):1025-1030.

Rautava L, Lehtonen L, Peltola M, Korvenranta E, Korvenranta H, Linna M, et al. The effect of birth in secondary- or tertiary-level hospitals in Finland on mortality in very preterm infants: a birth-register study. Pediatrics 2007 Jan;119(1): e257-63.

Redline RW. Placental inflammation. Semin. Neonatol. 2004 Aug;9(4):265-274.

Redline RW, O'Riordan MA. Placental lesions associated with cerebral palsy and neurologic impairment following term birth. Arch.Pathol. Lab.Med. 2000 Dec;124(12):1785-1791.

Redline RW, Wilson-Costello D, Borawski E, Fanaroff AA, Hack M. Placental lesions associated with neurologic impairment and cerebral palsy in very low-birth-weight infants. Arch.Pathol.Lab.Med. 1998 Dec;122(12):1091-1098.

Rees S, Inder T. Fetal and neonatal origins of altered brain development. Early Hum.Dev. 2005 Sep;81(9):753-761.

Reiss AL, Kesler SR, Vohr B, Duncan CC, Katz KH, Pajot S, et al. Sex differences in cerebral volumes of 8-year-olds born preterm. J.Pediatr. 2004 Aug;145(2):242-249.

Resch B, Radinger A, Mannhalter C, Binder A, Haas J, Muller WD. Interleukin-6 G(-174)C polymorphism is associated with mental retardation in cystic periventricular leucomalacia of preterm infants. Arch.Dis.Child.Fetal Neonatal Ed. 2009 Jan 15.

Rivera-Chavez FA, Peters-Hybki DL, Barber RC, O'Keefe GE. Interleukin-6 promoter haplotypes and interleukin-6 cytokine responses. Shock 2003 Sep;20(3):218-223.

Rocha G, Proenca E, Quintas C, Rodrigues T, Guimaraes H. Chorioamnionitis and brain damage in the preterm newborn. J.Matern.Fetal. Neonatal Med. 2007 Oct;20(10):745-749.

Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labour and delivery. Semin.Fetal. Neonatal Med. 2006 Oct;11(5):317-326.

Rorke-Adams L, Larroche J-C, Encha-Razavi F, de Vries L. Central nervous system. In: Gilbert-Barness E. Potter's Pathology of the fetus, infant and child. Second edition. Mosby Elsevier Inc; 2007. pp. 1959-2054.

Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet 2008 Jan 19;371(9608):261-269.

Schluter B, Raufhake C, Erren M, Schotte H, Kipp F, Rust S, et al. Effect of the interleukin-6 promoter polymorphism (-174 G/C) on the incidence and outcome of sepsis. Crit.Care Med. 2002 Jan;30(1):32-37.

Schmithorst VJ, Wilke M, Dardzinski BJ, Holland SK. Correlation of white matter diffusivity and anisotropy with age during childhood and adolescence: a cross-sectional diffusion-tensor MR imaging study. Radiology 2002 Jan;222(1):212-218.

Schneider JF, Il'yasov KA, Hennig J, Martin E. Fast quantitative diffusion-tensor imaging of cerebral white matter from the neonatal period to adolescence. Neuroradiology 2004 Apr;46(4):258-266.

Schultz C, Temming P, Bucsky P, Gopel W, Strunk T, Hartel C. Immature anti-inflammatory response in neonates. Clin.Exp.Immunol. 2004 Jan;135(1):130-136.

Shah DK, Anderson PJ, Carlin JB, Pavlovic M, Howard K, Thompson DK, et al. Reduction in cerebellar volumes in preterm infants: relationship to white matter injury and neurodevelopment at two years of age. Pediatr. Res. 2006 Jul;60(1):97-102.

Shalak LF, Laptook AR, Jafri HS, Ramilo O, Perlman JM. Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. Pediatrics 2002 Oct;110(4):673-680.

Shin KK, Jang Y, Koh SJ, Chae JS, Kim OY, Park S, et al. Influence of the IL-6 -572C>G polymorphism on inflammatory markers according to cigarette smoking in Korean healthy men. Cytokine 2007 Aug;39(2):116-122.

Singh KB, Gupta AK, Mann SB, Narang A. Comparative ABR profile in high risk infants. Auris Nasus Larynx 1998 May;25(2):143-148.

Skogstrand K, Hougaard DM, Schendel DE, Bent NP, Svaerke C, Thorsen P. Association of preterm birth with sustained postnatal inflammatory response. Obstet.Gynecol. 2008 May;111(5):1118-1128.

Skranes J, Vangberg TR, Kulseng S, Indredavik MS, Evensen KA, Martinussen M, et al. Clinical findings and white matter abnormalities seen on diffusion tensor imaging in adolescents with very low birth weight. Brain 2007 Mar;130(Pt 3):654-666.

Slattery MM, Morrison JJ. Preterm delivery. Lancet 2002 Nov 9;360(9344):1489-1497.

Snook L, Paulson LA, Roy D, Phillips L, Beaulieu C. Diffusion tensor imaging of neurodevelopment in children and young adults. Neuroimage 2005 Jul 15;26(4):1164-1173.

Soper DE, Mayhall CG, Froggatt JW. Characterization and control of intraamniotic

infection in an urban teaching hospital. Am J Obstet Gynecol. 1996 Aug;175(2):304-309

Speer EM, Gentile DA, Zeevi A, Pillage G, Huo D, Skoner DP. Role of single nucleotide polymorphisms of cytokine genes in spontaneous preterm delivery. Hum.Immunol. 2006 Nov;67(11):915-923.

Spinillo A, Capuzzo E, Stronati M, Ometto A, De Santolo A, Acciano S. Obstetric risk factors for periventricular leukomalacia among preterm infants. Br.J.Obstet.Gynaecol. 1998 Aug;105(8):865-871.

Srinivasan L, Dutta R, Counsell SJ, Allsop JM, Boardman JP, Rutherford MA, et al. Quantification of deep gray matter in preterm infants at term-equivalent age using manual volumetry of 3-tesla magnetic resonance images. Pediatrics 2007 Apr;119(4):759-765.

Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. JAMA 2004 Nov 17;292(19):2357-2365.

Stonek F, Metzenbauer M, Hafner E, Philipp K, Tempfer C. Interleukin 6-174 G/C promoter polymorphism and pregnancy complications: results of a prospective cohort study in 1626 pregnant women. Am J Reprod Immunol. 2008 Apr;59(4):347-351.

Sun H, Zhang D, Zhao J. The interleukin-6 gene -572G>C promoter polymorphism is related to intracranial aneurysms in Chinese Han nationality. Neurosci.Lett. 2008 Jul 25;440(1):1-3.

Suppiej A, Franzoi M, Vedovato S, Marucco A, Chiarelli S, Zanardo V. Neurodevelopmental outcome in preterm histological chorioamnionitis. Early Hum.Dev. 2009 Mar;85(3):187-189.

Szelenyi J. Cytokines and the central nervous system. Brain Res.Bull. 2001 Mar 1;54(4):329-338.

Taga T, Fukuda S. Role of IL-6 in the neural stem cell differentiation. Clin.Rev.Allergy Immunol. 2005 Jun;28(3):249-256.

Talar-Wojnarowska R, Gasiorowska A, Smolarz B, Romanowicz-Makowska H, Kulig A, Malecka-Panas E. Clinical significance of interleukin-6 (IL-6) gene polymorphism and IL-6 serum level in pancreatic adenocarcinoma and chronic pancreatitis. Dig.Dis.Sci. 2009 Mar;54(3):683-689.

Tasci Y, Dilbaz B, Uzmez Onal B, Caliskan E, Dilbaz S, Doganci L, et al. The value of cord blood interleukin-6 levels for predicting chorioamnionitis, funisitis and neonatal infection in term premature rupture of membranes. Eur. J.Obstet.Gynecol.Reprod.Biol. 2006 Sep-Oct;128(1-2):34-39.

Taudorf S, Krabbe KS, Berg RM, Moller K, Pedersen BK, Bruunsgaard H. Common studied polymorphisms do not affect plasma cytokine levels upon endotoxin exposure in humans. Clin. Exp.Immunol. 2008 Apr;152(1):147-152.

Tauscher MK, Berg D, Brockmann M, Seidenspinner S, Speer CP, Groneck P. Association of histologic chorioamnionitis, increased levels of cord blood cytokines, and intracerebral hemorrhage in preterm neonates. Biol Neonate. 2003;83(3):166-170.

Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J.Biol. Chem. 2000 Jun 16;275(24):18138-18144.

Thompson DK, Warfield SK, Carlin JB, Pavlovic M, Wang HX, Bear M, et al. Perinatal risk factors altering regional brain structure in the preterm infant. Brain 2007 Mar;130(Pt 3):667-677.

Thompson DK, Wood SJ, Doyle LW, Warfield SK, Lodygensky GA, Anderson PJ, et al. Neonate hippocampal volumes: prematurity, perinatal predictors, and 2-year outcome. Ann.Neurol. 2008 May;63(5):642-651.

Tischendorf JJ, Yagmur E, Scholten D, Vidacek D, Koch A, Winograd R, et al. The interleukin-6 (IL6)-174 G/C promoter genotype is associated with the presence of septic shock and the ex vivo secretion of IL6. Int.J.Immunogenet. 2007 Dec;34(6):413-418.

Tommiska V, Heinonen K, Ikonen S, Kero P, Pokela ML, Renlund M, et al. A national short-term follow-Up study of extremely low birth weight infants born in Finland in 1996-1997. Pediatrics 2001 Jan;107(1):E2.

Valkama AM, Tolonen EU, Kerttul LI, Paakko EL, Vainionpaa LK, Koivist ME. Brainstem size and function at term age in relation to later neurosensory disability in high-risk, preterm infants. Acta Paediatr. 2001 Aug;90(8):909-915.

Vallieres L, Campbell IL, Gage FH, Sawchenko PE. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. J.Neurosci. 2002 Jan 15;22(2):486-492.

Van Naarden K, Decoufle P. Relative and attributable risks for moderate to profound bilateral sensorineural hearing impairment

associated with lower birth weight in children 3 to 10 years old. Pediatrics 1999 Oct;104(4 Pt 1):905-910.

Vangberg TR, Skranes J, Dale AM, Martinussen M, Brubakk AM, Haraldseth O. Changes in white matter diffusion anisotropy in adolescents born prematurely. Neuroimage 2006 Oct 1;32(4):1538-1548.

Vasileiadis GT, Gelman N, Han VK, Williams LA, Mann R, Bureau Y, et al. Uncomplicated intraventricular hemorrhage is followed by reduced cortical volume at near-term age. Pediatrics 2004 Sep;114(3):e367-72.

Vergani P, Locatelli A, Ratti M, Scian A, Zangheri G, Pezzullo J, et al. Predictors of adverse perinatal outcome in twins delivered at < 37 weeks. J.Matern.Fetal.Neonatal Med. 2004 Dec;16(6):343-347.

Vergani P, Patane L, Doria P, Borroni C, Cappellini A, Pezzullo JC, et al. Risk factors for neonatal intraventricular haemorrhage in spontaneous prematurity at 32 weeks gestation or less. Placenta 2000 May;21(4):402-407.

Verma U, Tejani N, Klein S, Reale MR, Beneck D, Figueroa R, et al. Obstetric antecedents of intraventricular hemorrhage and periventricular leukomalacia in the low-birth-weight neonate. Am J Obstet Gynecol. 1997 Feb;176(2):275-281.

Vermont Oxford Network Database: The 2007 Annual NICU Quality Management Report for VLBW Infants. Vermont Oxford Network; Burlington, Vermont: 2008.

Virkola K. The lateral ventricle in early infancy. Doctoral thesis, 1988, Helsinki, Finland

Vermont Oxford Network Database. Manual of Operations for Infants Born in 2006. Release 10.0. Vermont Oxford Network 2005. Burlington, Vermont.

Vohr BR, Widen JE, Cone-Wesson B, Sininger YS, Gorga MP, Folsom RC, et al. Identification of neonatal hearing impairment: characteristics of infants in the neonatal intensive care unit and well-baby nursery. Ear Hear. 2000 Oct;21(5):373-382.

Volpe JJ. Cerebral white matter injury of the premature infant-more common than you think. Pediatrics 2003 Jul;112(1 Pt 1):176-180.

Weeks JW, Reynolds L, Taylor D, Lewis J, Wan T, Gall SA. Umbilical cord blood interleukin-6 levels and neonatal morbidity. Obstet.Gynecol. 1997 Nov;90(5):815-818.

Wen LZ, Xing W, Liu LQ, Ao LM, Chen SH, Zeng WJ. Cytomegalovirus infection in pregnancy. Int.J.Gynaecol.Obstet. 2002 Nov;79(2):111-116.

Whitelaw A. Intraventricular haemorrhage and posthaemorrhagic hydrocephalus: pathogenesis, prevention and future interventions. Semin. Neonatol. 2001 Apr;6(2):135-146.

Wilson-Costello D, Friedman H, Minich N, Siner B, Taylor G, Schluchter M, et al. Improved neurodevelopmental outcomes for extremely low birth weight infants in 2000-2002. Pediatrics 2007 Jan;119(1):37-45.

Winter CD, Pringle AK, Clough GF, Church MK. Raised parenchymal interleukin-6 levels correlate with improved outcome after traumatic brain injury. Brain 2004 Feb;127(Pt 2):315-320.

Wu CM, Ng SH, Liu TC. Diffusion Tensor Imaging of the Subcortical Auditory Tract in Subjects with Long-Term Unilateral Sensorineural Hearing Loss. Audiol.Neurootol. 2009 Jan 15;14(4):248-253.

Wu YW, Escobar GJ, Grether JK, Croen LA, Greene JD, Newman TB. Chorioamnionitis and cerebral palsy in term and near-term infants. JAMA 2003 Nov 26;290(20):2677-2684.

Yanowitz TD, Jordan JA, Gilmour CH, Towbin R, Bowen A, Roberts JM, et al. Hemodynamic disturbances in premature infants born after chorioamnionitis: association with cord blood cytokine concentrations. Pediatr.Res. 2002 Mar;51(3):310-316.

Yoon BH, Jun JK, Romero R, Park KH, Gomez R, Choi JH, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. Am J Obstet Gynecol. 1997 Jul;177(1):19-26.

Yoon BH, Romero R, Moon J, Chaiworapongsa T, Espinoza J, Kim YM, et al. Differences in the fetal interleukin-6 response to microbial invasion of the amniotic cavity between term and preterm gestation. J.Matern.Fetal.Neonatal Med. 2003 Jan;13(1):32-38.

Yoon BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, et al. The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6 concentration, amniotic fluid infection, and neonatal sepsis. Am.J.Obstet. Gynecol. 2000 Nov;183(5):1124-1129.

Yoshikawa S, Ikeda K, Kudo T, Kobayashi T. The effects of hypoxia, premature birth, infection, ototoxic drugs, circulatory system and congenital disease on neonatal hearing loss. Auris Nasus Larynx 2004 Dec;31(4):361-368.

Yung A, Poon G, Qiu DQ, Chu J, Lam B, Leung C, et al. White matter volume and anisotropy in preterm children: a pilot study of neurocognitive correlates. Pediatr.Res. 2007 Jun;61(6):732-736.

Zhang YT, Zhang CY, Zhang J, Li W. Age-related changes of normal adult brain structure: analysed with diffusion tensor imaging. Chin. Med.J.(Engl) 2005 Jul 5;118(13):1059-1065.