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THE ANTENATAL CAUSES OF CEREBRAL PALSY – GENETIC AND VIRAL ASSOCIATIONS

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INTRODUCTION

Cerebral palsy is the most common neurological disorder in children. Epidemiological evidence suggests that antenatal origins are a major cause. Currently there is no antenatal test for cerebral palsy, no proven preventable measures in late pregnancy, and no known cure. Cerebral palsy affects not only the diagnosed child, but also their family and the community, requiring considerable social and financial resources to assist these children in their daily lives.

Cerebral Palsy is a complex, multifactorial disorder, with many different definitions.[1] A recent definition of cerebral palsy states that *cerebral palsy describes a group of disorders of the development of movement and posture that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, cognition, communication, perception and/or behaviour, and/or by a seizure disorder*.[2] Cerebral palsy is characterised by non-progressive, abnormal control of movement or posture, and is not always diagnosed until months, or even years after birth.[3] Damage to the upper motor neurons of the brain[1,4] results in excessive muscular tonus, spasticity with increased stretch reflexes, and hyperactive tendon reflexes, all of which are often present in cases of cerebral palsy.[1]

Despite improvements in obstetric care over the last 50 years, the frequency of cerebral palsy in most communities has not decreased[3,5–9], and may have increased

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slightly as a result of the increased survival rate of infants of very low birth weight.[10–12] Caesarean delivery rates have also increased dramatically (up to 6-fold) without any apparent effect on cerebral palsy rates. Approximately 2 to 2.5 in every 1000 children born are diagnosed with cerebral palsy, making it the most common physical disability in childhood.[3,8,9,13] In contrast, there have been substantial decreases in both perinatal and maternal mortality,[3,9] suggesting that poor obstetric care is not a major cause of cerebral palsy, as had been previously thought.[3,6,14–17] Epidemiological studies indicate that only about 10% of cerebral palsy cases show possible signs of intrapartum fetal compromise, which may have had recent or longer-standing origins.[5,6,18,19] A recent audit by our group used the 2003 American College of Obstetricians and Gynecologists/American Academy of Pediatrics criteria to identify acute intrapartum hypoxia in the maternal and paediatric case notes of 213 cases of cerebral palsy from a single Australian tertiary care centre born between 1986–2003.[20] This audit identified major antenatal or paediatric cerebral palsy-related pathologies in 98.1% of all these cases, and that an isolated acute intrapartum hypoxic event was likely in only 2 of 46 neonates born at term and none born preterm.[20]

There are many risk factors for the occurrence of cerebral palsy, including maternal and antenatal factors such as prematurity, intrauterine infection, fetal coagulation disorders, multiple pregnancy, antepartum haemorrhage, breech presentation and chromosomal or congenital abnormalities.[5,6,8,9,14,21–25] This is consistent with other findings showing that infants with cerebral palsy only occasionally have strong evidence of primary asphyxia at birth.[26] However, the fetus with established brain dysfunction or susceptibility factors may sometimes experience secondary hypoxia at birth, ie acute on chronic brain injury. In retrospect no definitive cause can be identified in more than 75% of cases of cerebral palsy,[27] and data indicate that most children diagnosed with cerebral palsy did not have primary intrapartum asphyxia.

The risk of cerebral palsy is strongly associated with prematurity, occurring 20–30 times more often in infants weighing less than 1500g at birth.[28] Very preterm infants (<32 weeks) represent only 2% of all births but represent 25% of all children with cerebral palsy.[29] Multiple births are also a major risk factor, with twins (especially monochorionic) at higher risk of developing cerebral palsy than singletons.[30] The risks of having a child with cerebral palsy are 0.2%, 1.3% and 7.6% for singleton, twin and triplet pregnancies, respectively, and even higher when a co-fetus dies in utero.[30] Nevertheless, at least 50% of all diagnosed cases of cerebral palsy are in term or near-term infants.[31,32]

This review describes three new potential antenatal risk factors for cerebral palsy, namely inherited thrombophilic polymorphisms, cytokine polymorphisms and exposure to viral infections.

INHERITED THROMBOPHILIAS

The coagulation cascade is the body's response to a breach in the vascular system, which is normally balanced by both procoagulation and anticoagulation mechanisms.

However, there are instances in which this balance is altered, to favour either procoagulation or anticoagulation. Thrombophilia favours procoagulation and is an inherited or acquired condition that predisposes individuals to thromboembolism. Common inherited thrombophilias include the factor V Leiden (FVL) mutation causing activated protein C resistance, the prothrombin gene mutation (PGM), homozygosity or compound heterozygosity for one or both of the common polymorphisms at positions 677 and 1298 in the gene for 5, 10-methylenetetrahydrofolate reductase (MTHFR) associated with hyperhomocysteinaemia, and plasminogen activator inhibitor-1 (PAI-1) gene mutation. These inherited thrombophilic conditions predispose to the formation of thrombosis by impairing the natural coagulation pathway, by promoting excessive coagulation, by impairing anticoagulation, or by impairing the fibrinolytic pathways.[33] Pregnancy results in major haemostatic changes involving decreasing anticoagulation and increasing coagulation such that with the progression of pregnancy the overall homeostatic balance of coagulation is altered toward hypercoagulability.[34]

Endothelial cells play an active role in the coagulation response. When injured, endothelial cells synthesise and release tissue factor, which initiates coagulation via the extrinsic pathway of the coagulation cascade. Endotoxin and tumour necrosis factor (TNF) are among the substrates capable of inducing increased endothelial tissue factor activity.[35,36] TNF is also capable of attenuating the antithrombotic role of thrombomodulin, by promoting its endocytosis and degradation, and also inhibiting its transcription. In this way, coagulation is increased, while anticoagulation is decreased, leading to an overall shift towards a procoagulant state. Given that most thrombophilias require another risk factor to express the adverse phenotype, the presence of inflammatory cytokines (perhaps upregulated in response to infection) in conjunction with an inherited thrombophilia may provoke the development of thrombosis.

Accumulating epidemiological evidence implicates thrombosis, as well as inflammation, as important factors in the development and causation of cerebral palsy. One proposed vascular mechanism of cerebral palsy causation is that dislodged thrombi from the placental circulation may travel to the fetus via the umbilical vein, ductus venosus, and inferior vena cava, before crossing the patent foramen ovale to reach the fetal cerebral circulation.[37] The potential for widespread damage from lodgement of the thrombus and blockage of the fetal circulation to the brain is obvious. Such vascular occlusion could lead to white matter damage such as periventricular leukomalacia, with subsequent development of cerebral palsy.

The association between the tendency for thrombophilia and cerebral palsy has been the focus of a number of studies,[9,38–41] with FVL shown to be present in 26% of cerebral palsy cases and only 1.5% of controls in the study by Nelson and colleagues.[9] In 1998, it was proposed that undiagnosed thrombophilias, both inherited and acquired, of the mother and/or fetus, may be responsible for thrombosis in the maternal and/or fetal circulation, subsequently resulting in adverse pregnancy outcomes, such as cerebral palsy.[37] There is evidence suggestive of a relationship between cerebral palsy and placental infarcts, often related to spiral artery thromboses,[37,42–44] with one study identifying thrombi in fetal vessels of the placenta in 11 of 15 infants with cerebral

Table I Significant odds ratios and 95% confidence intervals for cerebral palsy for specified fetal inherited thrombophilic polymorphisms[41]

Thrombophilia	Gestational age (weeks)	Zygosity	Type of Cerebral Palsy	Odds Ratio (95% CI)
MTHFR C677T	All	Heterozygous	Diplegia	1.58 (1.02–2.45)
	32–36	Homozygous	All types of CP	2.55 (1.12–5.74)
		Heterozygous		1.91 (1.01–3.66)
	<32	Homozygous	Diplegia	2.76 (1.21–6.12)
MTHFR A1298C	32–36	Heterozygous	Diplegia	0.16 (0.02–0.70)
FVL	<32	Homozygous		9.12 (0.86–53.71)
		Homozygous vs heterozygous	Quadriplegia	26.00 (1.09–1551.59)
MTHFR + PGM	All	Homozygous + heterozygous	Quadriplegia	5.33 (1.06–23.25)

MTHFR = methylenetetrahydrofolate reductase gene, FVL = factor V Leiden, PGM = prothrombin gene mutation.

palsy.[43] However, the literature is by no means conclusive. One study investigating the link between cerebral palsy and thrombophilia suggested that, in patients with hemiplegic cerebral palsy, thrombophilia in itself was not an important causative factor.[45] The authors of this study did, however, postulate that the pathogenesis of cerebral palsy may be related to the interactions between thrombophilias and other risk factors for cerebral palsy, including maternal and/or neonatal infections[46] and cytokines.[47]

In both term and near-term infants, major causes of cerebral palsy include intrauterine infection and fetal or neonatal stroke.[16] A number of studies have investigated associations between cerebral palsy and the factor V Leiden mutation, with many cases classified as cerebral palsy caused by fetal or neonatal stroke.[9,38,42,48,49] It has been shown that 40% of cerebral palsy can be clearly identified with vascular factors, such as infarction and haemorrhage,[50] suggesting that thrombophilic polymorphisms, such as the factor V Leiden mutation, may play important aetiological roles in cerebral palsy.[39]

Since then, a large cerebral palsy case-control study has reported that MTHFR C677T approximately doubles the risk of cerebral palsy in preterm infants, and that this risk is dependent on the gestational age and cerebral palsy subtype of the infant (Table 1).[41] It appears that these thrombophilia results are only seen in preterm cerebral palsy infants – no associations were observed between any type of cerebral palsy and thrombophilia for children born ≥37 weeks gestational age. An additional finding was the protective effect displayed by the carriage of MTHFR A1298G for diplegic cerebral palsy (Table 1).[41] This is postulated to be the result of homozygosity for MTHFR A1298G protecting against having the MTHFR C677T polymorphism, probably by increasing the early embryonic loss rates.[51]

MTHFR C677T can result in hyperhomocysteinaemia, particularly in conditions of folate or vitamin B12 deficiency. Hyperhomocysteinaemia may exert thrombophilic

effects by altering the normal antithrombotic phenotype of the endothelium, enhancing the activities of factors XII and V, as well as depressing the activation of protein C, and also by recruiting leukocytes and augmenting leukocyte-induced endothelial cell activation.[52,53] Mild-to-moderate hyperhomocysteinaemia, with and without the C677T polymorphism, has been linked to arterial disease and venous thromboembolism.[54,55] The thrombophilic tendency associated with hyperhomocysteinaemia could in itself lead to neonatal stroke, but it appears unlikely that this mechanism could explain an increased risk of diplegic cerebral palsy in very preterm infants.[56] Preterm birth is a final common pathway of a variety of pathophysiologic conditions. The association between infection and spontaneous preterm labour is now well established, and is thought to be responsible for up to 40% of cases, with an especially strong association with very preterm birth.[57,58] Recent studies have shown that any inflammatory damage to the vascular lining could be augmented in a hyperhomocysteinaemic environment by recruiting more leukocytes and aggravating the leukocyte-induced endothelial damage.[55,59,60] The significant positive association between the polymorphic variant at MTHFR C677 and very preterm infants with cerebral palsy makes an additive adverse interaction between infection and mild-to-moderate increases in circulating homocysteine an attractive hypothesis deserving further study.

Homozygous Factor V Leiden was observed to increase the risk for quadriplegia in very preterm infants, and there was also a 26 fold increased risk of developing quadriplegia when homozygosity for factor V Leiden was compared with heterozygosity (Table 1).[41] A non-significant trend for heterozygous FVL to be negatively associated with quadriplegia in very preterm infants was also observed. Carrying one abnormal FVL allele may protect the vulnerable fetal brain from the more major degrees of intraventricular haemorrhage by increasing the clotting ability of the infant, and/or convey some protection in severe infectious processes.[61]

CYTOKINE POLYMORPHISMS

Cytokines, present in the amniotic fluid, are risk markers of neonatal brain damage and subsequent long term disability.[62] During the course of intrauterine infection, inflammatory cytokines play a pivotal role in the pathogenesis of brain white matter damage and the subsequent development of cerebral palsy.[47] An association has been demonstrated between cytokine concentrations from umbilical cord blood and the development of brain white matter lesions,[47] and high expression of TNF alpha (TNF-α) has been demonstrated in neonatal brains with periventricular leukomalacia.[63] However, it remains unclear whether the cytokines themselves mediate the damage, cause the damage, or whether the infection itself is responsible for the damage. A cytokine hypothesis has been postulated, the so called "Fetal Inflammatory Response Syndrome" (FIRS), indicating that cytokines act as a final common pathway for injury to the central nervous system, and that they may be initiated by a number

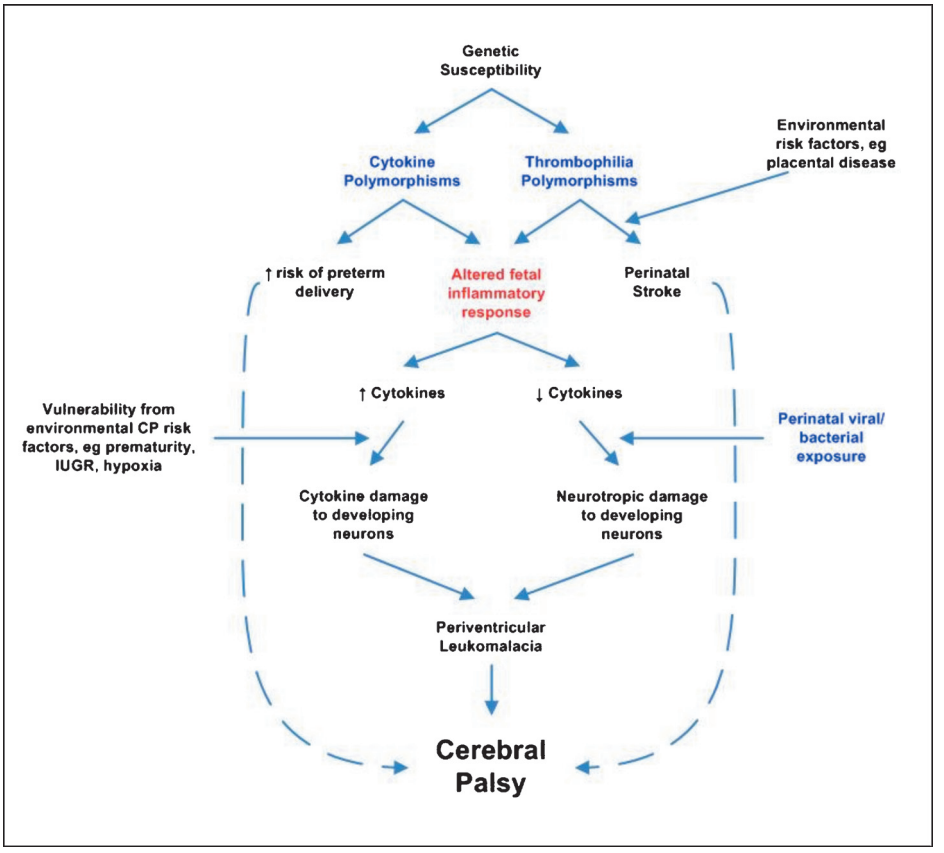

Figure 1 Hypothetical schematic diagram illustrating possible causes of cerebral palsy.

of different insults, including infection, hypoxic-ischaemic injury, reperfusion injury, and toxin-mediated injury.[64,65] Finally, the role of proinflammatory cytokines in preterm birth has been extensively investigated, demonstrating strong associations between preterm birth and the presence of proinflammatory cytokines.[66–71] Preterm birth is a recognised risk factor for the development of cerebral palsy, suggesting that the FIRS plays a major pathogenetic role in both preterm birth and cerebral palsy. Figure 1 is a hypothetical diagram illustrating possible causes of cerebral palsy.

A large number of studies have investigated the association between cytokine responses to infection and the development of periventricular leukomalacia and/or cerebral palsy,[9,17,47,62,66,67,72–82] as increased levels of cytokines in the amniotic fluid and fetal circulation appear to increase the risks for both neonatal brain injury and long-term disability.[62] A recent study by Urakubo and colleagues[83] demonstrated that TNF-α levels were significantly increased in the placenta when pregnant rats were injected with lipopolysaccharide (LPS), suggesting that proinflammatory cytokine levels are increased in the fetal environment in response to maternal infection,

which may significantly impact upon the developing brain. These studies suggest that cytokines are markers of infection and possible brain damage, and may contribute to this brain damage via unidentified pathways.

TUMOUR NECROSIS FACTOR-α

Tumour necrosis factor alpha (TNF-α) is a 17kDa protein made up of 157 amino acids. This proinflammatory cytokine is produced in response to infection.[84] It mediates a variety of biologic processes, including growth, development and immune responses of the brain,[85] and is produced by a range of cells, including macrophages, haematogeneous and neural cells.[86] Its main biological function, however, is its ability to recognise many pathogens and act quickly, promoting a broad range of immunological and inflammatory responses.[84] An excessive cytokine response may pose more of a risk than the infection. If the production of TNF-α is excessive, and is released systemically in large quantities, fatal complications such as multiple organ failure may occur.[84]

TNF-α is directly toxic to neurons and may cause white matter damage associated with periventricular leukomalacia through its cytotoxic effect, damaging oligodendrocytes.[78] TNF-α may also disturb developmental transitions from the oligodendrocyte precursor to the mature oligodendrocyte.[87] Cells positive for TNF-α were identified in the white matter in 9 of 13 periventricular leukomalacia cases when infant post-mortem tissues were stained with antibodies against TNF-α, suggestive of damage caused by cytokines in the white matter regions of the brain.[79] In a separate study, infants suffering periventricular leukomalacia had increased TNF-α and IL-6 in brain tissue, as well as evidence of lymphocytes infiltrating the brain.[78] TNF-α also exerts a powerful prothrombotic effect, one of the main mechanisms being the increased expression of tissue factor.

A study by Yoon et al[78] found that the expression of TNF-α was more common with periventricular leukomalacia (88% of cases) than without periventricular leukomalacia (18% of cases). Concentrations of TNF-α in amniotic fluid are higher in patients with intrauterine infection and subsequent preterm labour.[75,80] Proinflammatory cytokines in the brain are higher in infants who died with periventricular leukomalacia than in infants with no evidence of white matter damage.[47] Higher levels of proinflammatory cytokines were also measured in the amniotic fluid of preterm infants who later developed cerebral palsy, and in term infants just days after birth, compared to infants who did not develop cerebral palsy (both preterm and term).[9,88] These studies support a role for an excessive cytokine fetal inflammatory response leading to periventricular leukomalacia.

About 60% of the variation in TNF-α production is genetically determined.[89] The TNF-2 polymorphism is located in the promoter region of the gene, and is a single nucleotide G→A base pair substitution at nucleotide −308 relative to the transcriptional start site.[84] This polymorphism has been associated with high

Table 2 Significant odds ratios and 95% confidence intervals for TNF-α−308 polymorphism and cerebral palsy[93]

Type of Cerebral Palsy	Gestational age (weeks)	TNF-α−308 Zygosity	Odds Ratio (95% CI)
Hemiplegia	<32	Homo- or heterozygous	2.38 (1.02–5.58)
Quadriplegia	≥37	Heterozygous	1.82 (1.04–3.15)

TNF-α = tumour necrosis factor alpha.

levels of TNF-α.[90–92] In our studies, carriage of the TNF-α-308 polymorphism was associated with an increased risk of cerebral palsy, particularly for the development of quadriplegia in term-born babies (OR 1.82, 95% CI 1.04–3.15) (Table 2).[93] These results suggest that this polymorphism, which has previously been associated with high circulating TNF-α levels, is detrimental to the fetus and can directly or indirectly cause brain damage. The combination of increased levels of TNF-α due to the polymorphism and the normal physiologic upregulation of TNF-α as a result of infection may contribute to the pathogenesis of white matter damage and subsequent cerebral palsy.

MANNOSE BINDING LECTIN

Mannose-binding lectin (MBL) is a serum protein involved in the activation of the complement system of the innate immune system and is involved in the fetal inflammatory response to injury and infection. The MBL gene has 6 known polymorphic sites,[94] which together result in haplotypes associated with high (HYPA), intermediate (LYQA, LYPA), low (LXPA) and defective (HYPD, LYQC, LYPB) levels of circulating MBL.[95]

The first report of associations between recurrent infections and low levels of MBL was in 1989.[96] Since then, there have been many papers published investigating the associations between MBL efficiency and increased susceptibility to infection. It is important to note that 90% of MBL deficient individuals do not acquire repeated infections, possibly due to the redundancy of the complement system.[97] It can be postulated that the phenotypical manifestation of MBL deficiency is only apparent when combined with another immunodeficiency, either acquired or genetically determined. It has been shown that MBL deficient children presenting with recurrent infection also had concomitant or transient IgG subclass deficiency.[98] Because the MBL pathway plays an important role in eliminating pathogens, especially in neonates and infants,[99] fetuses deficient in MBL may be more susceptible to subclinical infections and/or inflammatory events *in utero*.[100] Decreased levels of MBL resulting from polymorphisms in this gene may contribute to the pathogenesis of cerebral palsy via a decreased response to neurotropic viruses or other infections.

Table 3 Significant odds ratios and 95% confidence intervals for associations between cerebral palsy and specified MBL haplotypes. Results are compared against the wild-type HYPA haplotype.[104] Viruses investigated included enteroviruses; herpes simplex viruses 1 and 2; Epstein-Barr virus; cytomegalovirus; varicella-zoster virus; and human herpesviruses 6, 7, and 8

Infection Status	Type of Cerebral Palsy	Haplotype	Gestational Age (weeks)	Odds Ratio (95% CI)
Not considered	All Cerebral Palsy	LYPA	All	1.57 (1.00–2.46)
			<37	2.43 (1.41–4.18)
			<32	2.54 (1.34–4.76)
	Diplegia	LYPA	32–36	3.70 (0.80–13.72)
	Hemiplegia	LYPA	<37	2.77 (1.02–7.26)
			<32	4.48 (1.55–12.65)
	Quadriplegia	HYPD	All	3.47 (1.41–8.31)
			≥37	3.07 (0.86–8.90)
			<37	4.13 (0.95–13.74)
			<32	7.86 (1.67–29.48)
Negative viral exposure	Diplegia	LYQA	<37	0.31 (0.08–0.91)
Positive viral exposure	All Cerebral Palsy	LYPA	<32	3.67 (0.91–12.56)
	Hemiplegia	LYPA	<32	8.25 (1.08–52.76)
	Quadriplegia	LYQA	≥37	3.56 (1.27–10.30)
		HYPD	All	5.24 (1.03–21.67)
			<32	18.33 (2.06–150.68)

Mutations in the MBL gene are strongly associated with children presenting to hospitals with infection, and these mutations increase susceptibility to infection in children who are heterozygous or homozygous for the mutations.[101] This increased risk of infection may be of greatest importance when immune responses are either immature (such as in the neonate) or defective. Recent reports have described associations between low-producing MBL alleles and disease severity in conditions in which immunity is already significantly impaired.[102,103] Low-producing alleles of MBL may also influence the clinical phenotype of other immunodeficiency diseases.[94]

Only one study has investigated the role of MBL haplotypes in the development of cerebral palsy.[104] Our large case-control study found that MBL haplotype LYPA was associated with all types of cerebral palsy (OR 1.57, 95% CI 1.00–2.46), and also with preterm hemiplegic cerebral palsy (OR 2.77, 95% CI 1.02–7.26) (Table 3).[104] The defective HYPD haplotype was associated with quadriplegic cerebral palsy (OR 3.47, 95% CI 1.41–8.31). Furthermore, when subanalysis on samples previously testing positive for exposure to viral infection was conducted, similar patterns of significance were observed, whereas analysis on samples negative for exposure to viral infection showed no positive associations.[104] This suggests that both genetic susceptibility factors and exposure to viral infection are necessary to significantly increase the

risk of cerebral palsy. It also suggests that the associations between MBL haplotypes and cerebral palsy are mediated through modifications of the immune response to infection. Further research is required to investigate these hypotheses.

VIRAL INFECTION AND THE FETAL RESPONSE TO INFECTION

FIRS is described as a multisystem disorder that may result in preterm delivery and/or neurological complications, such as cerebral palsy.[105] Histopathological evidence of this inflammatory response is present when funisitis (inflammation of the umbilical cord) and chorioamnionitis are confirmed. It is characterised by increased levels of proinflammatory blood cells in the amniotic fluid and the accumulation of inflammatory blood cells (polymorphonuclear leukocytes) in the umbilical cord and chorion.[106] These have been associated with increased risks of both neonatal infection and the development of cerebral palsy.[107,108] The fetal inflammatory response to intra-amniotic infection is biologically important, even more so than the maternal inflammatory response.[109] A multicentre cohort of 1078 infants of birthweight <1500 g showed that, in preterm births, abnormal fetal inflammatory responses contribute to cerebral white matter damage and that fetal responses to maternal infection can damage the fetal brain without the presence of fetal brain infection.[109] Evidence is also accumulating for the fetal role in chorioamnionitis. In the past, chorioamnionitis has been assumed to represent a maternal infection,[110] but it is now apparent that it is primarily a fetal inflammatory response.[28] The onset of spontaneous preterm labour with preterm premature rupture of membranes is preceded by a systemic proinflammatory cytokine response in the fetus, which is probably the fetal response to the presence of microbial products.[111] Antenatal infection and brain white matter damage appear to be linked by the FIRS.[62] Umbilical vein plasma concentrations of interleukin-6, a cytokine implicated in the regulation of the host response to infection, are increased in neonates born to mothers with clinical chorioamnionitis,[112] also suggesting that the inflammatory process causing chorioamnionitis is a fetal response.

In addition to the FIRS, intrauterine infection is postulated to be an important contributor to the development of cerebral palsy, and is currently the focus of intensive research. To date, many studies have investigated the role of infection in the development of cerebral palsy by using surrogate markers of infection, such as chorioamnionitis and maternal pyrexia. Chorioamnionitis can be described as histopathological evidence of infection, characterised by an inflammatory leukocyte infiltration of the chorion and amnion. Chorioamnionitis can make an infant of very low birth weight more vulnerable to neurologic damage[113] and increase the risk of cerebral palsy.[114–116] Also, a combination of maternal chorioamnionitis and neonatal seizures identifies infants of very low birth weight who are at increased risk of cerebral palsy.[115,117] Further evidence for the role of intrauterine infection in brain damage and cerebral palsy comes from an experiment which induced intrauterine infection in rabbits by injecting doses of *Escherichia coli* bacteria. This experiment showed that

bacterial infection can lead to fetal white matter damage in the brain,[118] providing more evidence for the role of intrauterine infection as a cause of cerebral palsy. A meta-analysis investigated the potential association between chorioamnionitis and cerebral palsy in both full term and preterm infants.[119] This meta-analysis has since been revisited and now includes studies published in 2000.[120] Clinical chorioamnionitis was found to be significantly associated with cerebral palsy (relative risk 1.9, 95% CI, 1.5–2.5), and also cystic periventricular leukomalacia (relative risk 2.6, 95% CI, 1.7–3.9), demonstrating that chorioamnionitis is a risk factor for both cerebral palsy and cystic periventricular leukomalacia.[120] A more recent case-control study in extremely low birthweight infants has also demonstrated strong associations between cerebral palsy and chorioamnionitis, both clinical and histological (OR 3.71, 95% CI 1.16–11.9). Intrauterine exposure to maternal infection has also been associated with an increased risk of long-term neurologic morbidity.[14] A study by Yanowitz and colleagues[121] in 2002 found that chorioamnionitis was associated with increased cytokine concentrations in cord blood and that infants with fetal vessel inflammation had higher levels of proinflammatory cytokines. The risk of brain injury in premature infants born after chorioamnionitis is apparently increased when cerebrovascular inflammation and systemic vasculitis are both present.[121] Maternal infection is associated with white matter damage, periventricular leukomalacia, and long-term neurological dysfunction and cerebral palsy. It is still not clear if the brain damage is secondary to the proinflammatory cytokine response to the initial infection.[17,122] Infection remote from the brain results in the release of products of infection into the circulation. These products may cross the blood-brain barrier, either as a result of immaturity of this barrier,[123] or due to release of proinflammatory cytokines, which impair its integrity.[69,77] Having penetrated the blood-brain barrier, these products of infection, which include proinflammatory cytokines such as TNF-α, can cause damage to developing white matter, in the form of periventricular leukomalacia. This white matter damage may result from a number of different mechanisms, including direct tissue damage, stimulation of fetal microglia to produce more TNF-α,[105] and disruption of the endothelium and/or ependyma.[77]

During pregnancy, maternal resistance to some viral infections is decreased, subsequent to depression of cell-mediated immunity with consequent reactivation of latent virus or increased susceptibility to primary infection (if non-immune). As such, the fetus may be at risk of transplacental virus transmission.[124] There are many viruses capable of causing damage to the brain, in particular the developing brain, and these are referred to collectively as neurotrophic viruses.[125] Many neurotrophic viruses are capable of causing brain damage in the human fetus. The list includes rubella virus, cytomegalovirus, varicella zoster virus, enteroviruses, adenoviruses, rotaviruses, and other members of the herpesvirus group.[126] More recently the arenavirus (lymphocytic choriomeningitis virus) has been added to the list.[127]

For a pathogen to cause fetal damage *in utero*, it must cross the placenta or fetal membranes. This may occur during the viraemic, bacteraemic, or parasitic phase of maternal infection.[128] The placenta acts as a potential barrier to maternal/fetal infection. However this barrier may be less effective in early pregnancy during its

Table 4 Significant odds ratios and 95% confidence intervals for associations between exposure to perinatal viral exposure and cerebral palsy

Virus	Gestational Age (weeks)	Type of Cerebral Palsy	Odds Ratio (95% CI)
Herpes PCR Group B	All	All Cerebral Palsy	1.68 (1.09–2.59)
		Diplegia	1.93 (1.03–3.61)
		Hemiplegia	2.07 (1.10–3.88)
	≥37	Diplegia	2.45 (1.02–5.89)
		Hemiplegia	2.38 (1.15–4.92)
	<37	Quadriplegia	2.87 (1.09–7.59)
Any herpesvirus	≥37	All Cerebral Palsy	1.52 (1.09–2.13)
Any virus	All	All Cerebral Palsy	1.64 (1.17–2.28)
	≥37	All Cerebral Palsy	1.30 (1.00–1.67)

Herpes PCR Group B = varicella-zoster virus, human herpesvirus −6 or −7; Any herpesvirus = herpes simplex viruses 1 and 2, Epstein-Barr virus, cytomegalovirus, varicella-zoster virus, human herpesvirus −6, −7 or −8; Any virus = any herpesvirus or enterovirus.

development, and potentially when the placenta is damaged by vascular disease, e.g., infarction. Placental dysfunction has been associated with thrombophilia, systemic lupus erythematosus (SLE), diabetes, chronic hypoxia, and severe preeclampsia. Disruption of the placental barrier may increase the vulnerability of the fetus to maternal infection. In addition, gestational age at the time of maternal infection plays an important role in the development of fetal infection. Although fetal transmission is much higher in infections contracted in the third trimester, exposure to fetal infection in the first or early second trimester is more likely to cause severe fetal damage and miscarriage.[128] This gestational age effect may explain in part why not all patients with potentially damaging infections go on to develop severe neurological sequelae such as periventricular leukomalacia and cerebral palsy because the brain may be more vulnerable during specific periods of fetal or neonatal brain development. Cytomegalovirus, herpes simplex viruses, varicella zoster virus, adenovirus, and enterovirus are all capable of crossing the placenta and infecting the fetus.[129–136] The likelihood of maternal infection resulting in infection of the fetus varies according to the specific virus, whether the infection is primary or recurrent, and the gestational age of the fetus at the time of infection. Once the infection has crossed the placenta into the fetal circulation, there is the potential for damage, both by the infectious agent directly, and also by the FIRS. Some viruses can persist for months or years after the initial infection.[137–144] These viruses may have effects as long as 30 years after the original infection.[138,139,142–144]

Our large retrospective case control study of cerebral palsy cases and controls identified associations between perinatal exposure to neurotropic viruses and cerebral palsy.[145] In particular, the detection of Herpes PCR Group B viruses (Varicella zoster virus, human herpesvirus 6 or human herpesvirus 7) increased the risk of developing cerebral palsy, with an OR of 1.68 (95% CI 1.09–2.59) (Table 4). These findings suggest

the possibility of complex relationships between exposure to viral infections and cerebral palsy development, and are worthy of further investigation.

FUTURE RESEARCH

Most of the associations between cerebral palsy and thrombophilia, cytokine polymorphisms and perinatal viral exposure in our studies have been derived from analyses of a large retrospective case-control cohort and has involved multiple comparisons. In some subanalyses, numbers of cases have been small. Therefore, type I and II errors are possible. This necessitates larger prospective studies where there is specific *a priori* testing of the hypothesised associations. Such a large study is underway in Australia and will help confirm or refute these risk factors as possible causative factors in cerebral palsy. The collection of more clinical data and known clinical risk factors such as multiple pregnancy, infections, fetal growth restriction etc will allow better assessment of the interaction of known environmental cerebral palsy risk factors and these possible new genetic risk factors.

PREVENTION

The current understanding of these possible genetic susceptibility factors for cerebral palsy and their interaction with environmental risk factor such as viral exposure in pregnancy supports the "double or triple jeopardy" hypothesis where a combination of risk factors at different gestations can lead to the different types of neuropathology that in turn lead to the different types of cerebral palsies. Confirmation of this hypothesis may lead to preventative strategies. Large scale immunisation is effective in reducing adverse effects of infection with viral agents, as evidenced by the rubella virus vaccine reducing the teratogenic effects of rubella during pregnancy. Children are already being immunised against varicella-zoster virus, and it will be of interest to observe if there is a decline in adverse pregnancy outcome rates, including cerebral palsy, in the next generation following the vaccination of their mothers. The introduction of vaccines for a wide range of viruses and bacteria would reduce the risk of women contracting infection during pregnancy, and therefore minimise the risk of transplacental transfer of infection to the fetus. Antiviral therapies, if their safety can be established, may also play a role in reducing the risk of adverse outcomes.

The reported associations between inherited thrombophilic and cytokine polymorphisms and adverse outcomes need to be confirmed in further large-scale studies. There remain many more candidate polymorphisms worthy of investigation, in order to fully understand any potential interactions between these genetic factors and the subsequent development of adverse pregnancy outcomes. If any of these polymorphisms are shown to be clinically relevant, intervention strategies can be implemented to reduce the risk of these polymorphisms contributing to adverse

events. Although still controversial, antithrombotic therapy may be useful to women identified as having an increased risk of thrombosis. Fetal genetic screening *in utero* of these high-risk women may also be useful. It should be emphasized that current antithrombotic therapy using low-molecular weight heparins will potentially only "protect" the uteroplacental circulation and will not protect the fetus from (theoretical) adverse effects of fetoplacental thrombosis. Perhaps novel antithrombotic strategies such as ultra-low molecular weight heparins and other direct antithrombins may be able to prevent fetoplacental thrombosis without the extremely adverse consequences seen with the use of warfarin. Fetal genetic screening *in utero* for cytokine polymorphisms such as TNF-α-308 and polymorphisms in the MBL gene may also provide clinicians with more clinically relevant treatment options. Prophylactic antiviral or antibiotic treatment may be considered during pregnancy for women with cytokine polymorphisms capable of altering the immune response to infection. Lastly, if our ongoing studies show maternal polymorphisms are involved, then prenatal gene therapy may be a future goal for prevention.

CONCLUSIONS

Research into the antenatal causes of cerebral palsy has shown that inherited thrombophilias, inherited cytokine polymorphisms and viral infections are all associated with the subsequent development of cerebral palsy. Future research should investigate interactions between genes and the environment, which may create double or multiple jeopardy for cerebral palsy. Possible preventative strategies should be explored, including vaccination programmes against neurotropic viruses identified as being associated with cerebral palsy. This research also has medico-legal and political implications. The possible causal pathways for most cerebral palsy outcomes currently cannot be influenced by obstetric practice. Determination of the antenatal causes of cerebral palsy, with the possible advent of prenatal screening, may lead to its eventual prevention, saving much human suffering and saving hundreds of millions of dollars annually.

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