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Genetics of perinatal brain injury in the preterm infant

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1. ABSTRACT

Due to developmental immaturity of the central nervous system, effects of an adverse intrauterine environment and need for intensive care postnatally, preterm infants are at high risk of sustaining brain injury in the perinatal period. Infants who suffer brain injury in the perinatal period are at risk for long-term neurodevelopmental sequelae. Clinical and experimental data supports a significant role for inflammatory mediators in the pathophysiology of perinatal brain injury. Abnormalities in coagulation proteins in the sick preterm newborn may accentuate the risk for intraventricular hemorrhage. Polymorphisms in TNFα, IL-1β, IL-4, IL-6 and IL-10 as well as mutations in coagulation proteins have been investigated as potential candidate genes to modify risk and or severity of perinatal brain injury. Preliminary evidence suggests a role for cytokine genes as risk modifiers for IVH and PVL.

2. INTRODUCTION

Premature infants are at high risk of brain injury during the perinatal period. The unique vulnerability of these infants is due to developmental immaturity of the central nervous system, effects of an adverse intrauterine environment and need for intensive care postnatally. The two major forms of brain injury sustained in the perinatal period by premature infants are intraventricular-periventricular hemorrhage (IVH) and white matter damage commonly described as periventricular leukomalacia (PVL). Although IVH and PVL are distinct pathological entities, they often occur in combination. The risks for these injuries are strongly related to gestation and birth weight, with the most immature infants at highest risk (1, 2). The incidence of these injuries may be as high as 40% in the most immature infants (depending on definition used) (1, 2). These perinatal brain injuries, particularly PVL, frequently result in long-term neurodevelopmental disabilities (3). Recent studies point to potential genetic

contributions to the development of these complications. Most of the studies to date examine the role genetic variation of inflammatory mediators and coagulation proteins on IVH and/or PVL and these will be the focus of this review.

3. PATHOGENESIS OF PERINATAL BRAIN INJURY

The pathogenesis of IVH and PVL are generally thought to be distinct, although there are some common elements. For example, cerebral hypoperfusion is a common antecedent of both lesions. More recently it has become apparent that the pathophysiology of these lesions may have important differences. Disturbances in cerebral hemodynamics with increased variability of cerebral blood flow may play a greater role in the development of IVH, whereas cerebral hypoperfusion, exposure to infection antenatally and subsequent increased production of inflammatory mediators may be the dominant mechanisms in PVL (4).

3.1. Intraventricular hemorrhage

The primary pathological lesion in IVH is bleeding from small vessels in the germinal matrix. The premature infant is susceptible to bleeding in this region because of developmental, anatomical, and hemodynamic factors. The germinal matrix is metabolically very active and is supplied with a rich capillary network (5). However, vasculature of the germinal matrix is immature with thin vessels, poorly supported by the surrounding interstitium. Experimental and clinical data suggest that alterations in cerebral hemodynamics play a significant role in the pathogenesis of IVH. In animal models systemic hypertension (with or without antecedent hypotension) produces bleeding into the germinal matrix (6). In preterm infants, systemic hypotension results in injury to the vessels of the germinal matrix and leads to impairment of cerebral autoregulation. Upon restoration of systemic blood pressure, cerebral blood flow increases which results in rupture of germinal matrix vessels. This pathogenesis is supported by the clinical observations that the cerebral circulation in sick preterm infants is pressure passive and fluctuations of systemic blood pressure (and hence cerebral perfusion) are associated with the development of IVH (7-9). Additionally, increased fibrinolytic activity and decreased concentrations of clotting factors in preterm infants may exacerbate the degree of bleeding that occurs during the reperfusion (10, 11).

3.2. Periventricular leukomalacia

The premature infant is at high risk to develop PVL because of a tendency towards hypoperfusion with subsequent ischemia of the periventricular white matter and the sensitivity of the developing oligodendrocytes in this region to injury (12, 13). Both anatomical and physiologic factors underlie the tendency for hypoperfusion of the periventricular white matter. The vascular supply of the periventricular white matter is relatively underdeveloped in the very premature infant which results in a border zone(s) between the perforating medullary arteries (14, 15). In addition to, or as a consequence of, the developmental anatomy, blood flow to the cerebral white matter is extremely low in the preterm newborn (16). Thus the periventricular white matter is exquisitely sensitive to even minor drops in cerebral perfusion pressure.

The other major factor in the pathogenesis of PVL is sensitivity of oligodendrocyte precursors to injury. The neuropathology of PVL is characterized by injury to oligodendrocyte progenitors with subsequent disrupted myelination (17, 18). Oligodendrocyte precursors are dominant in the cerebral white matter between 23-32 weeks gestation (19). There are several mechanisms by which oligodendrocyte injury may occur. These include glutamate excitotoxicity, free radical and cytokine mediated injury, and oxidant stress (20-26). For additional information on the pathogenesis and clinical features of these conditions the readers are referred to several excellent reviews of the subject (3, 12, 27, 28).

3.3. Role of inflammation in perinatal brain injury

Clinical and experimental data supports a greater role for inflammation and inflammatory mediators in the pathophysiology of perinatal brain injury then previously appreciated. Animal models of brain injury, human autopsy studies, clinical observations and measurement of inflammatory mediators all support a fundamental role for inflammation in the development of perinatal brain injury. Inflammatory mediators may be upregulated in the CNS as a consequence of ischemia-reperfusion injury in purely asphyxiated premature infants. However, up-regulation of inflammatory mediators and subsequent brain injury frequently occur in infants who are not obviously asphyxiated but who are exposed to clinical or subclinical infection (29, 30). Experimental models of intrauterine infection are associated with focal white matter cysts that mimic PVL (31-34). Direct administration of endotoxin intracerebrally causes white matter injury in neonatal animals (35). Further, in contrast to systemic hypoxia-ischemia, endotoxin administration results in selective white matter injury (33). Additionally, endotoxin exposure may increase vulnerability to hypoxic injury (36). Clinical evidence for this pathogenesis is supported by the finding that intrauterine infection of the placenta (chorioamnionitis) or umbilical cord (funisitis) is associated with the development of IVH and PVL (29, 37-41). The risk of injury appears to be related to the severity of the placental infection, with severe funisitis being associated with the greatest risk (40).

3.3.1. Inflammatory mediators in animal models of brain injury

Animal models utilizing hypoxia-ischemia and endotoxin injury provide insight into the pathogenesis of perinatal brain injury (Table 1). These models support a role for inflammation in the pathogenesis of perinatal brain injury. The primary source of inflammatory mediators in the CNS is probably glial cells (microglia) although other cell types are capable of expressing inflammatory mediators (42, 43). Microglial cells constitutively and in response to stimuli express a large variety of cytokines and their receptors (44, 45). Exposure to endotoxin or β amyloid significantly increased the expression of tumor

necrosis factor- α (TNFα), interleukin (IL)-6, IL-8, IL-10, IL-12, IL-15, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α and MIP-1β (45). Like other immune cells microglia express the endotoxin receptor (Toll-like receptor 4: TLR4) and activation of TLR4 is necessary for endotoxin induced neuronal injury (46). In experimental models of hypoxia-ischemia injury proinflammatory cytokines (TNFα, IL-1β and IL-6) are upregulated in both adult and neonatal animals (47-50). Similarly, in endotoxin induced brain injury TNFα, IL-1β and IL-6 are upregulated (35, 51).

TNFα plays a dual role in brain injury, being involved in acute injury but also having some neuroprotective functions (52). Blocking TNFα with either neutralizing antibodies or soluble TNF-receptor-I (sTNF-RI) reduces the extent of hypoxic-ischemic brain injury (53-55). Direct intracerebral injection of TNFα induces activation of microglia, and vasogenic edema (56, 57). In contrast, TNF receptor (p55) deficiency exacerbates hypoxic-ischemic injury (58, 59). Additionally, pretreatment with TNFα (intracisternally) reduces the extent of hypoxic-ischemic brain injury (60). It is also possible that TNFα is deleterious in some sites in the CNS while protective in others (61). These data suggest that although the increased expression of TNFα in the ischemic period may be deleterious, this cytokine may play a role in facilitating recovery after brain injury.

A large body of evidence implicates IL-1β as a key mediator in hypoxic-ischemic brain injury. Intracerebral injection of IL-1β in the neonatal rat induces activation of microglia and CNS cell apoptosis to a much greater degree than TNFα (56). Administration of IL-1β increases experimental hypoxic-ischemic injury while neutralizing antibodies to IL-1β, inhibitors of interleukin-1β converting enzyme (ICE) or administration of interleukin-1 receptor antagonist (IL-1RA: an endogenous antagonist of IL-1) reduce injury (48, 62-66). Genetically modified mice with over expression of IL-1RA, deficiency of ICE, or deficiency of IL-1 receptor-1 (IL-1R1) have amelioration of ischemic brain injury (49, 67-71). Mice lacking IL-1β (or IL-1α) have similar degree of brain injury to wild type, while double knockouts have dramatically reduced injury (72). This probably represents compensation by other components of the IL-1 system to chronic depletion of either IL-1β or IL-1α. The exact mechanisms by which IL-1β and TNFα causes brain injury are incompletely understood. IL-1β and TNFα stimulate production of other cytokines, endothelial adhesion molecules and nitric oxide in the CNS (55, 73-76).

The role of IL-6 in hypoxic-ischemic brain injury is complex. IL-6 is an essential cytokine for normal brain function under normal conditions as well as during injury (77). IL-6 is upregulated during hypoxic-ischemic injury but many of its effects are neuroprotective. These effects may be secondary to inhibition of IL-1β and stimulation of IL-1RA and soluble TNFR-1 (p55) (78). Administration of IL-6 significantly decreases experimental hypoxic-ischemic injury and increased parenchymal concentrations are associated with improved outcome in adult traumatic brain injury (79-81). IL-6 deficient animals have an impaired inflammatory response, increased oxidative stress and neurodegeneration following direct brain lesioning (82). In contrast, chronic over expression of IL-6 in transgenic mice resulted in decreased neurogenesis (83).

Anti-inflammatory cytokines such as IL-10 may have a neuroprotective role in the CNS. IL-10 inhibits IL-1β and endotoxin induced cytokine production by glial cells (44, 45, 75). A neuroprotective role for IL-10 is suggested by studies in which IL-10 knockout mice develop greater infarct volumes following middle cerebral artery occlusion than wild type similarly treated (84). Further, treatment with IL-10 ameliorates brain injury following focal and global ischemic insults (85-87).

Chemotactic cytokines (chemokines) have been shown to be involved in the pathogenesis of brain injury in animal models. MCP-1, a proinflammatory chemokine, attracts monocytes to the tissue where it is expressed. MCP-1 expression is upregulated in neurons and glial tissues in animal models of ischemia-reperfusion, traumatic, excitotoxic and endotoxin induced brain injury (88-92). MCP-1 likely plays an important role as over expression of MCP-1 exacerbates ischemic brain injury and MCP-1 deficiency reduces the injury (93, 94). Other chemokines (MIP-1α, MIP-1β, RANTES, IP-10) are upregulated during brain injury but the role and importance of these changes have yet to be determined (89, 92, 95). Chemokine receptor (CCR3, CCR5) expression is variable being unchanged in some models of brain injury and increased in others (95).

The role of inflammatory mediators in brain injury is complicated by the observations that while increased expression of these cytokines during the early stage of injury is harmful, low levels of these same cytokines enhance neuronal regeneration during the recovery stage after injury (96).

3.3.2. Clinical evidence of role of inflammatory mediators in development of perinatal brain injury

Although less direct than for animal models, clinical and pathological evidence has linked inflammatory mediators to brain injury in preterm infants (Table 2, Table 3). Pathological examination of the brain of infants who have died with PVL show increased *in situ* expression of several cytokines and their receptors. Expression of TNFa, IL-6, IL-1β, IL-2 and IL-2R is increased in postmortem brain tissue (microglia and astrocytes) from infants with PVL compared to control infants (97-100). CSF concentrations of IL-6, TNFα, and IL-10 are increased in infants who have significant white matter damage (PVL, severe ventriculomegaly) (101).

Several studies have linked prenatal infection and elevation of cord and amniotic fluid cytokines to perinatal brain injury. Increased amniotic fluid and/or umbilical cord concentrations of TNFa, IL-6, IL-10, IL-18 and IL-1β are associated with the development of PVL (102-107). Similarly, cord blood IL-1ß, IL-6, and IL-8 concentrations are significantly increased in infants who subsequently

Table 1. Role of inflammatory mediators in experimental brain injury

Mediator	Effect in animal model	References
TNFα	Increased infarct size in ischemia-reperfusion injury	35
IL-1β	Increased infarct size in ischemia-reperfusion injury	35,49
IL-1RA	Protective	35
IL-6	Protective/ Chronic overexpression harmful	79,81,82,83
IL-10	Protective	84,85,86
IL-18	increased expression in hypoxic-ischemic injury	197,198
MCP-1	Increased infarct size in ischemia-reperfusion injury	93,94

Table 2. Evidence of role for inflammatory mediators in development of white matter disease in human preterm infants

Mediator		References
TNFα	Amniotic fluid and CSF concentrations increased, *in situ* expression is increased in brains of infants with PVL	98,99,199 97, 101,103
IL-1β	Amniotic fluid concentrations increased, *in situ* expression increased in brains of infants with PVL	99,103,199
IL-2	IL-2 and IL-2R *in situ* expression is increased in brains of infants with PVL	100
IL-6	Cord blood, amniotic fluid and CSF concentrations increased, *in situ* expression is increased in brains of infants with PVL	97,101,103,104,106,107
IL-10	CSF concentrations increased	101
IL-18	Cord blood concentrations increased	105

Table 3. Evidence of role for inflammatory mediators in development of intraventricular hemorrhage

Mediator		References
IL-1β	Cord blood concentrations increased	108,109
IL-6	Cord blood and amniotic fluid concentrations increased	107,108,110
IL-8	Cord blood concentrations increased	108

Table 4. Evidence of role for inflammatory mediators in pathogenesis of cerebral palsy in preterm infants

Mediator		References
IL-2	Increased cord concentrations in infants who develop CP	112
IL-6	Increased amniotic fluid concentrations in infants who develop CP	103
IL-8	Increased amniotic fluid and cord blood concentrations in infants who develop CP	103,111
IL-18	Increased cord concentrations in infants who develop CP	105

developed IVH (108-110). Furthermore, perinatal infection, cord blood and amniotic fluid concentrations of pro-inflammatory cytokines are correlated with adverse longer-term outcomes such as cerebral palsy (Table 4) (41, 103, 105, 111, 112).

4. GENETIC FACTORS IN THE DEVELOPMENT OF PERINATAL BRAIN INJURY

4.1. Cytokine gene polymorphisms

Recent evidence suggests that functional cytokine gene variants that result in altered production of inflammatory cytokines (TNFα, IL-6 and IL-1β) or anti-inflammatory (IL-10) may modify disease processes. Since the expression of TNFα, IL-6 and IL-1β are critically linked to the pathogenesis of brain injury and subsequent repair, genetic variants of these and other cytokines are candidates to modify risk for brain injury in the preterm infant. Several recent studies provide preliminary evidence to suggest that variation in TNFα, IL-6 and IL-1β genes may influence the development of IVH and or PVL in preterm infants (Table 5).

4.1.1. TNFa

As we have seen, TNFα is a central mediator of brain injury. Thus it is reasonable to suspect that genetic variants of the TNFα gene may modify risk for similar injuries in the perinatal period. The TNFα gene is polymorphic, containing numerous polymorphisms in the promoter region (113-120). Although controversial, a biallelic (G to A) single nucleotide polymorphism (SNP) at position -308 in the TNFa promotor region is associated with high (A) or low (G) gene transcription *in vitro* (113-116). Other polymorphisms may also have functional consequences, especially in different ethnic groups (121). More recent studies suggest that TNFα promoter polymorphisms are nonfunctional (122, 123). It is possible that TNFα promoter polymorphisms may serve as markers for neighboring gene polymorphisms (CD14 or lymphotoxin-α) that may influence disease susceptibility (124, 125).

We examined the role of the TNFα -308 GA SNP on outcome in ventilated very low birth weight infants (126). In the 169 infants who could be evaluated for IVH

Table 5. Cytokine Gene variants and perinatal brain injury

Cytokine /Polymorphism	Associations	Reference
TNFα -308A	Increased risk of IVH/increased risk of neurodevelopmental impairment	126,127
IL-1β -511T	Increased risk of IVH and PVL	141
IL-1RA VNTR	No association	141
IL-6 –174G	Decreased Risk of IVH	153
IL-4 –590T	Increased risk of severe IVH (African-American infants)	170
IL-10 –1082A	Increased risk of PVL	168,169
MCP-1	No association	126

Table 6. Interaction of the IL-1 –511T allele and tracheal isolation of *Ureaplasma urealyticum* and perinatal brain injury

	UU –ve No –511T	UU –ve With IL-1 -511T	UU +ve No –511T	UU +ve With IL-511T	P value
IVH	5/40 (13)	38/127 (30)	2/18 (11)	23/66 (35)	0.027
IVH = Grade 3	4/40 (10)	28/127 (22)	0 (0)	45/66 (20)	0.064
PVL	2/40 (5)	5/127 (4)	1/18 (6)	11/66 (17)	0.014
IVH or PVL	5/40 (13)	40/127 (32)	3/18 (17)	27/66 (41)	0.010

Numbers in parenthesis represent percentages

and or PVL, the TNFα-308A allele was associated with a significant increase in the risk for IVH. In infants with the TNFα –308 A allele (AG and GG genotype groups combined) the incidence of IVH of all grades was 40% compared to 24% in infants with the GG genotype. The incidence of high grade IVH (Grades 3 and 4) and PVL were not different between groups in this study.

Other evidence suggests that extremely low birth weight (ELBW) infants with the TNFα –308 A allele are at increased risk of adverse neurodevelopmental outcome (127). In this group of infants, the TNFα –308 A allele was associated with increased risk for developing posthemorrhagic hydrocephalus. Further, MDI, PDI and NDI scores <70 occurred more often among infants with the TNFα –308 A allele. In this study, another polymorphism in the TNFa gene (-238GA), was not associated with neurodevelopmental outcome. Another study did not find any association of the TNFα -308GA, -238GA or –376GA polymorphisms and the development of CP (128).

The studies above are very heterogenous with respect to the ethnicity and selection of subjects, which may lead to differing conclusions as to the importance of the TNFα -308GA polymorphism. The TNFα -308GA polymorphism is more common in Caucasian (18 to 27%) than in African-American (10%) individuals (129-131). Further studies will be needed to define the role of TNFα polymorphisms in perinatal brain injury.

4.1.2. IL-1b and IL-1RA

Because of the experimental and clinical evidence implicating IL-1β as a key mediator in hypoxic-ischemic brain injury, polymorphisms of the IL-1 gene family may modify risk for similar injuries in the perinatal period. The magnitude of IL-1ß expression has a genetically determined component. SNPs at positions –511 (C to T) and –39 (T to C) are associated with increased IL-1ß production *in vivo* (-511T and –39C alleles). These two SNPs are in almost complete linkage disequilibrium with each other (the –39 CT SNP being the functional site) (132-134). The IL-1ß –39C or –511T alleles have been associated with increased risk for or disease progression in several inflammatory conditions (135). The biological effects of IL-1ß are opposed partially by another member of the IL-1 gene family. IL-1RA competes with IL-1ß for binding to the IL-1R but does not initiate signal transduction. Thus, biological effects of IL-1ß depend, at least partially, on the molar ratio of IL-1RA to IL-1ß (136). As with IL-1ß, the magnitude of IL-1RA expression has a genetic component. A penta-allelic polymorphism in intron 2 of the IL-1RA gene is thought to influence IL-1RA production (137, 138). This polymorphism consists of an 86 base pair (bp) variable number tandem repeat (VNTR). Allele 1 (common allele) has 4 copies of the 86 bp sequence while allele 2 has 2 copies, allele 3 has 5 copies, allele 4 has 6 copies and allele 3 has 3 copies. Allele 2 has been associated with increased production of IL-1RA *in vivo* and *in vitro* (137-139).

We recently presented preliminary data on the role of the IL-1β -511 CT and IL-1RA VNTR polymorphisms on outcome in 215 ventilated very low birth weight infants (140, 141). In this group of infants the IL-1β -511 T allele (increased the IL-1β production) was associated with large increased in risk for IVH and PVL. IVH occurred in 33% of infants with the T allele (CT and TT genotype groups) and 14% in infants with only the C allele (OR3.0; 95% CI 1.4-6.4, p=0.003). Severe IVH was similarly increased in infants with the T allele. PVL was also increased mainly in infants with CT genotype.

Because of the known risk of chorioamnionitis on PVL, potential interactions of tracheal *Ureaplasma urealyticum* colonization (a marker for chorioamnionitis) and the IL-1β-511T allele on the incidence and severity of these complications were determined (141). Infants with both the IL-1β –511T allele and isolation of Uu were at significantly greater risk of PVL than infants with one or less of these risk factors (Table 6). These data suggests an interaction between environmental (Ureaplasma colonization/

chorioamnionitis), genetic (IL-1β –511T SNP) and developmental (gestation) factors in determining risk to develop PVL. There was no association between isolation of Uu and IVH; however IVH was associated with the IL-1β -511T allele (and gestation). In contrast to that seen for the IL-1β SNP there was no association between any IL-1RA VNTR genotype (allele) and development of IVH or PVL.

4.1.3. IL-6

IL-6 is a strong candidate gene to modify risk for perinatal brain injury. Several polymorphisms have been reported for the IL-6 gene (142-144). A functional G to C polymorphism at position -174 relative to the transcription start point was associated with lower expression using a gene reporter assay (145). This region was in the vicinity of binding sites for several transcription factors including NF-IL6 (144). The effects of this polymorphism, however, are more complex and may be stimulus dependent, cell line dependent and may be different *in vivo* than *in vitro*. *In vitro* IL-6 production in LPS stimulated mononuclear cells was higher in the CC genotype in newborn infants (146). Following coronary artery bypass surgery the C allele was associated with increased plasma IL-6, whereas following vaccination the G allele was associated with increased plasma IL-6 (147-149). This complexity is further compounded by additional functional polymorphisms that are in linkage disequilibrium with the -174 SNP (147, 148).

The IL-6 –174 GC SNP has been implicated as a risk modifier in several disease processes (145, 150-152). Harding et al have recently reported the role of the IL-6 –174 GC SNP in perinatal brain injury and neurodevelopmental outcome (153). They evaluated 151 surviving preterm infants for cranial US abnormalities and developmental outcome. Infants with the CC genotype (higher IL-6 production *in vitro*) were significantly at greater risk of perinatal brain injury. Both IVH and white matter disease were increased. Additionally, these injuries translated into an increased risk of neurodevelopmental disability at 2 years of age. In adult stroke patients, however, the GG genotype was associated with an increased severity of disease (154).

We have recently examined the role of the IL-6 –174 GC SNP on the incidence of IVH and PVL in our dataset (unpublished observations). Two hundred and eight-five ventilated very low birth weight (VLBW) infants were analyzed. We found no relation between IL-6 genotype and development of either IVH or PVL. The incidence of IVH was 28% in infants with the GG genotype, 26% in GC, and 13% in CC (p=0.602). Similarly the incidence of PVL was: GG 5.5%, GC 8.8% and CC 0%. Some of the differences between the 2 studies may reside in the ethnic background of the patients. Our infants were 80% African-American and 20% Caucasian. Ethnic background greatly influences the incidence of IL-6 –174C allele (as well as other cytokine gene polymorphisms) (142, 155, 156). The frequency of the IL-6 -174C allele is much lower in the African-American population (142). However, when stratified by ethnic background there was still no relationship between IL-6 genotype and brain injury.

4.1.4. IL-10

Although there are no studies describing a role for IL-10 in the development of IVH or PVL, IL-10 may be an important candidate gene to modify perinatal brain injury because of its neuroprotective function in animal models. The magnitude of IL-10 secretion is partially genetically determined by several polymorphisms in the promotor region (157, 158). The SNP at -1082 (G to A) is thought to be a major determinant of the magnitude of IL-10 secretion and may influence outcome in several disease states (159-161). The -1082 GA polymorphism lies with a putative Ets transcription site and is associated (A allele) with low IL-10 production *in vitro*. The IL-10 -1082 SNP is in linkage disequilibrium with 2 other SNPs (-819 C/T and -592 C/A) and appear in three haplotypes (157, 162, 163). The polymorphism at -819 (C/T) may affect an estrogen receptor element and the -592 (C/A) site lies within a region with negative regulatory function. The GCC haplotype (G at position -1082, C at position -819, C at -592) is associated with high IL-10 secretion, while the ACC and ATA haplotypes are associated with intermediate and low IL-10 secretion respectively (164). Other potentially functional polymorphisms in the IL-10 gene, most notably the -2849 AG SNP, have not been studied in the context of perinatal brain injury(165-167).

We examined the role of the IL-10 -1082 GA SNP on outcome in ventilated VLBW infants (168). In this study 283 ventilated VLBW infants had cranial US data and could be genotyped. There was no effect on the IL-10-1082 GA SNP on the incidence or severity of IVH or PVL. The incidence of IVH was 28% in the GG, 33 % in the GA and 22% in the AA genotypes. There was a non significant trend for less severe IVH in the AA genotype group (lowest IL-10 secretion group) in that IVH greater than grade 2 occurred in 11% of AA infants compared to 22 and 23% in GA and GG infants (p=0.065). PVL occurred in none of the GG infants and 7% of GA and 8% of AA infants.

Dordelmann et al reported on the potential role of IL-10 –1082 GA SNP on perinatal brain injury and developmental outcome in 73 children less than 32 weeks gestation (169). Infants with the GG genotype had a significant reduction in risk for white matter disease (any periventricular echodensity or echolucency on postnatal ultrasound). The IL-10 –1082 GG may also reduce the incidence of cerebral palsy and developmental delay among these infants.

4.1.5. Other cytokine polymorphisms

Numerous other cytokines potentially could play a role in determining risk for perinatal brain injury. We have examined the role of the MCP-1 –2518 GA and IL-4 –590 CT SNPs on outcome in ventilated VLBW infants (126, 170). In this group of predominately African-American infants there was no association of the MCP-1 –2518 GA SNP and risk for PVL or IVH.

Interleukin-4 is a pleiotropic cytokine that modulates cytokine production in astrocytes (75). Increased plasma concentrations of IL-4 are seen in cerebral infarction in adults (171). A genetic variant in the

Table 7. Coagulation factor variants and perinatal brain injury

Factor/Mutation	Association	Reference
Factor XIII Val34Leu	Decreased risk of white matter injury (Leu)/ Increased risk of IVH	186
Factor V Leiden	None vs. increased risk of IVH /decreased risk of IVH extension?	187,188,189,190
Factor II (Prothrombin) G20210A	None vs. increased risk of IVH /decreased risk of IVH extension?	187,188,189
MTHFR C677T	No association	188, 189,193

IL-4 (-590 C/T) gene is associated with increased IL-4 production (T allele) and thus is a further candidate gene to influence the development of perinatal brain injury in premature infants (172). In our population of ventilated VLBW infants the IL-4 –590 T allele was associated with an increased incidence of severe IVH (170). However, the effect was restricted only to African American infants.

4.2. Thrombophilias and coagulation gene mutations

Because of the role of hemostatic factors in the development of IVH, several investigators have examined mutations of coagulation proteins as possible risk modifiers for perinatal brain injury (Table 7). Several common mutations in coagulation proteins are associated with increased tendency to thrombotic events. Factor XIII stabilizes the fibrin clot increasing its resistance to fibrinolysis. Low factor XIII levels in the premature infant may be one of the factors that contributes to the relatively higher fibrinolytic activity that is associated with risk for IVH (10, 173). A mutation in a subunit of factor XIII consisting of a valine to leucine change in codon 34 results in increased activation (174). The factor XIII 34Leu allele has been associated with decreased incidence of ischemic events and increased hemorrhagic events in adults (175-178).

The factor V Leiden mutation is a common mutation in the Caucasian population associated with increased risk of thrombotic events (179). This mutation is caused by a G to A change in nucleotide 1691 of the factor V gene resulting in glutamine replacing arginine at position 506 (180). This mutation results in decreased inactivation of factor V leading to greatly increased thrombin generation (181). A G to A substitution at nucleotide 20210 in the 3' untranslated region of the prothrombin gene results in increased thrombin concentration and increases risk for thrombosis (182, 183).

Two mutations (C677T and A1298C) in the 5,10-methylenetetrahydrofolate reductase (MTHFR) lead to hyperhomocystinemia under conditions of decreased folate or vitamin B12 concentrations (184). These mutations are associated with an increased risk of thrombosis (185). Co-inheritance of more than one of the thrombophilias is associated with greater risk of thrombotic events than with a single thrombophilia.

The effect of the factor XIII Arg34Leu mutation on the incidence and severity of IVH and PVL in a large cohort of Caucasian VLBW infants has been reported by Gopel et al (186). In this cohort, the factor XIII 34Leu allele was significantly associated with a decreased risk of PVL. However, carriage of this mutant allele was associated with a trend for increased risk of IVH.

The role of the factor V Leiden mutation and risk of IVH or extension is unclear. In a small study of 28 infants with Grades II-IV IVH the incidence of the factor V Leiden mutation (all heterozygous) was increased compared to control infants (not significant) and the general population (187). The incidence of the factor V Leiden mutation was increased compared to control infants without IVH in another small study of 17 infants with IVH (Grades II-IV) (188). In both of these studies the association of factor V Leiden was not significant. In contrast, in a larger study of 305 infants carriage of either prothrombin G20210A or factor V Leiden mutation was associated with a reduced risk of extension of IVH but not a difference in the overall rate of IVH (189). Two additional studies have shown that the incidence of factor V Leiden is increased in preterm infants with Grade 1 IVH compared to control infants lending indirect support that prothrombotic mutations may reduce the risk of extension (190, 191). However in neither study were infants with higher grade IVH studied. The MTHFR C677T mutation was not associated with an increased (or decreased) risk of IVH in any of the studies to date (188, 189).

We examined the potential role of thrombophilic mutations and perinatal brain injury in a retrospective study of 99 ELBW infants. In contrast to the other studies, we examined a largely African-American population. Prothrombotic mutations are less frequent in this population (192). We found no association between the Factor V Leiden, prothrombin G20210A or MTHFR C677T mutations and the development of either IVH or PVL (193).

5. FUTURE DIRECTIONS

Studies to date provide us with preliminary evidence that genetic factors may play a role in determining the risk for and or the degree of perinatal brain injury. Additional studies are needed to confirm or refute any of the potential associations that have been seen, as single gene association studies are often not replicated in different populations. Lack of reproducibility may be in part due to differences in ethnicity in which different susceptibility genes may be involved. Additionally, other risk factors such as antenatal inflammation may differ between study populations.

Future studies will have to be designed to accommodate several important issues. First, the incidence

of single gene polymorphisms or mutations varies dramatically between ethnic groups (142, 156, 160, 192). This has two important considerations: study power and functional significance. Second, the definition of control groups will be important. Perinatal brain injury is strongly related to gestation at birth and control infants should be at a similar developmental risk of injury. Many of the genetic associations that potentially influence perinatal brain injury may also influence the risk of extreme prematurity (194). Thus an association between injury and a particular gene may reflect the risk of prematurity if controls are not matched appropriately. Third, definition of perinatal brain injury should be improved. Cranial ultrasound exams fail to detect many infants with significant white matter disease (195). Imaging modalities such as magnetic resonance imaging should be used to categorize degree and type of white matter injury. Alternatively (or additionally), developmental followup as in Harding's study will be needed to provide long-term effects of genetic factors on outcome (153). Other genes or other polymorphisms within the genes studied may be important and will need to be included in future studies. For example, variations in platelet glycoprotein IIb/IIIa have been associated with stroke (196). Finally, studies should be designed to assess the interactions between environmental factors such as perinatal and postnatal infection, birth asphyxia and maternal conditions.

Such studies obviously will need to be large and well funded. However, significant advances in the neurodevelopmental outcome of very preterm infants have not improved in the last decade to a significant extent and studies of these types may show what are the critical mediators in a complex pathophysiology. This information may lead to the development of newer more effective therapies to prevent or ameliorate the devastating effects of perinatal brain injury in the very preterm infant.

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Abbreviations: IL: interleukin; TNFα: tumor necrosis factor-α; MCP-1: monocyte chemoattractant protein-1, MIP: macrophage inflammatory protein; IVH: intraventricular hemorrhage; PVL: periventricular leukomalacia; MTHFR: 5,10-methylenetetrahydrofolate reductase; SNP: single nucleotide polymorphism; bp: base pair; VNTR: variable number of tandem repeats

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