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Role of Polymorphic Variants as Genetic Modulators of Infection in Neonatal Sepsis

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ABSTRACT: This study is a retrospective, case control study involving 535 preterm infants examining the roles of sequence polymorphisms in genes that mediate host immune responses to bacterial infection in newborn infants. A total of 49 single nucleotide polymorphisms (SNPs) in 19 candidate genes including inflammatory cytokines (*IL6*, *IL10*, *IL1B*, and *TNF*), cytokine receptors (*IL1RN*), toll-like receptors (*TLR2*, *TLR4*, and *TLR5*), and cell surface receptors (*CD14*) were genotyped. Subjects were stratified into three groups (sepsis, suspected sepsis, and control). The data were analyzed using a family-based transmission disequilibrium test. We found that birth weight, gestational age, duration of rupture of membranes, and presence of clinical chorioamnionitis were strongly associated with sepsis. Polymorphisms in *TLR2* (rs3804099), *TLR5* (rs5744105), *IL10* (rs1800896), and *PLA2G2A* (rs1891320) genes were associated with sepsis. Allelic variants in *PLA2G2A* and *TLR2* were associated with Gram-positive infections, whereas *IL10* was associated with Gram-negative infections ($p < 0.05$). We conclude allelic variations in *PLA2G2A*, *TLR2*, *TLR5*, and *IL10* may moderate the predisposition to sepsis in preterm infants. **(*Pediatr Res* 68: 323–329, 2010)**

Neonatal sepsis is a major cause of morbidity and mortality among newborn infants occurring in 1% of term and up to 20% of very low birth weight (VLBW) infants (1–3). The mortality rate varies from 3 to 50% in early-onset sepsis, and up to 40% in late-onset sepsis (2). Susceptibility factors for newborn infants to sepsis include maternal and environmental exposures, immune status, and inflammatory responses. These interacting factors can be modified by variation among individuals in gene function or expression that may have significant clinical implications.

The neonate is a unique host immunologically. Only a few studies have characterized the immune response of neonates to bacterial infection (4–8). Studies of the innate and adaptive immune responses in newborn infants, and in particular extremely preterm neonates, have suggested that there are both impaired neutrophil function and unique cytokine responses (5,6,8).

The pathophysiology of sepsis involves highly complex interactions between invading microorganisms, the innate and adaptive immune systems of the host, and multiple downstream events leading to organ dysfunction and death (9). Altered cellular signaling due to circulating cellular mediators contributes to dysregulation of immunity, tissue repair, and cellular stress responses (10). The typical initial response to bacterial infection is the recognition of pathogen-associated molecular patterns (PAMPs) *via* cell surface receptors. Toll-like receptor-2 (TLR-2) seems to be involved in recognition of Gram-positive bacteria and TLR-4 in the recognition of Gram-negative bacteria (11). IL1 receptor-associated kinase (IRAK) undergoes autophosphorylation after interaction with the TLR-MyD88 complex (12). Ultimately, this cascade results in the expression of specific cytokines. Proinflammatory mediators (IL-1B, IL-6, TNF-A, and LTA) activate host defenses against pathogens. IL-1 receptor antagonist (IL-1RA) antagonizes the proinflammatory action of IL-1B. IL-10 is also a potent anti-inflammatory mediator, limiting the inflammatory response, thus preventing an excessive reaction, which may itself cause organ damage and cell death (13).

Studies in twins suggest that host genetic factors significantly contribute to interindividual variation in susceptibility to infections (14,15). Genetic association studies have suggested that one or more candidate genes have a role in pathogenesis of sepsis (14,16). Recent evidence also supports the view that gene-environment interactions result in certain patients having heightened susceptibility to neonatal sepsis and provide insights into mechanisms of disease development (16–18).

Identification of genetic variations in the genes involved in bacterial-induced cellular response and those involved in the pathogenesis of sepsis may allow the development of new diagnostic tools, improved classification of sepsis, and more accurate predictors of patient outcomes. In this study, we examined the relationship between genetic variants in 19 genes involved in host responses to bacterial infection and sepsis in newborn infants.

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Abbreviations: BPI, bactericidal/permeability increasing protein; **IRAK,** interleukin-1 receptor-associated kinases; **LPS,** lipopolysaccharides; **MyD88,** myeloid differentiation protein 88; **PAMPs,** pathogen-associated molecular patterns; **PLA,** phospholipase A; **SNP,** single nucleotide polymorphism; **VLBW,** very low birth weight

METHODS

This study is a retrospective, case control study involving preterm infants admitted to the Neonatal Intensive Care Unit of the University of Iowa Children's Hospital from 2000 to 2007. The study was approved by the Institutional Review Board for Human Research at University of Iowa. Parental consent was required for inclusion.

Subjects. Three groups of preterm infants (<37 wk gestation) were defined in this study. The infected group (79 infants) consisted of those infants with symptoms and signs of sepsis: temperature instability, respiratory compromise, (increased oxygen requirement, respiratory distress, apnea, and cyanosis), cardiovascular compromise (bradycardia, tachycardia, poor perfusion, and hypotension), neurologic changes (hypotonia, lethargy, and seizures), and gastrointestinal compromise (feeding intolerance, abdominal distension, and vomiting). These infants had positive cultures for blood, cerebrospinal fluid (CSF), or urine and were treated with antibiotics for ≥7 d. The suspected sepsis group (254 infants) consisted of infants who had clinical signs of sepsis and received antibiotic treatment for ≥7 d but had sterile cultures for blood, CSF, or urine. The control group (202 infants) consisted of infants who never had a positive culture for blood, CSF, or urine and received no antibiotic therapy for ≥7 d at any time during their hospital stay. Exclusion criteria in this study removed infants with birth defects and from pregnancies affecting twins and higher multiples. Clinical and outcome data were abstracted from a Neonatal Intensive Care Database and the infants' medical records. DNA was collected as part of an ongoing study [institutional review board (IRB) approved] of diseases in newborn infants. DNA was extracted from cord blood or buccal swabs for infants and from venous blood or buccal swabs for the parents. One or both parents were enrolled in this study along with their infants.

Candidate genes. Nineteen candidate genes were selected for analysis including genes involved in the pathogenesis of inflammation and sepsis as well as in immune regulation. These genes encode pattern-recognition receptors (*CD14*, *TLR2*, *TLR4*, and *TLR5*), intracellular signaling proteins (*MyD88* and *IRAK1*), proinflammatory cytokines (*IL1B*, *IL1RN*, *IL6*, *TNF*, and *LTA*), antiinflammatory cytokines (*IL10*, and *IL10RA*), chemokines (*IL-8*), bactericidal-permeability increasing protein (*BPI*), angiotensin converting enzyme (*ACE*), mannose binding lectin-2 (*MBL2*), and phospholipase A2 (*PLA2G2A*). All are located on autosomes except *IRAK1*, which is located on the × chromosome. Most of the single nucleotide polymorphisms (SNPs) represented functional variants or tagging SNPs characterized through the International HapMap Project. Eight SNPs had been implicated in sepsis predisposition in other studies: *IL10* (rs1800872 and rs1800896), *IL1B (rs*1143643), *IL6 (rs*1800795), *MBL2* (rs5030737 and rs7096206), *CD14* (rs2569190), *BPI* (rs4358188) (17,19–21). The genes and SNPs investigated are listed in Table 1.

Genotyping. Genotyping for SNP markers was performed using the TaqMan genotyping system on an ABI 7900HT (Applied Biosystems, Foster City, CA). After PCR amplification, allele determination was detected by end-point analysis using SDS software. Forty-nine SNPs in the 19 genes were genotyped. Genotyped data were entered into a Progeny database (Progeny Software, LLC, South Bend, IN) for generation of datasets for analysis.

Statistical analysis. Nonrandom allele inheritance was assessed by transmission disequilibrium test (TDT) as implemented in the software Family Based Association Test (FBAT) (22,23). Alleles and genotypes at each marker were tested for association with sepsis. Haplotype analysis was performed for genes that had more than one SNP genotyped. Differences among the three study groups were evaluated for the following characteristics: 1) gender, type of delivery, duration of rupture of membranes, and clinical chorioamnionitis using χ^2 tests and 2) gestational age (GA), birth weight, days on mechanical ventilation, and total days on oxygen using tests of ANOVA with Tukey's adjustment for multiple tests at the $p = 0.05$ level. A multivariable logistic regression was conducted to adjust for GA in the TDT analysis. Principal component analysis was used to define a phenotype for TDT that was a composite of correlated data. Data are presented as mean with minimum and maximum values. The $p < 0.05$ was considered significant. Because this study is both hypothesis testing and hypothesis generating, less stringent p values are also of interest. The statistical software SAS (SAS 9.1.3, SAS institute Inc, Cary, NC) was used.

Table 1. *List of genes and SNPs*

Gene	Chromosome	SNP
IL10	1	rs1800872
		rs1800896
		rs2222202
TLR5	1	rs5744105
PLA2G2A2	1	rs1891320
		rs1891321
		rs955587
		rs2307246
IL1B	2	rs1143643
		rs1143634
		rs1143627
IL1RN	2	rs419598
		rs315952
MyD88	3	rs7744
		rs6853
IL8	4	rs4073
TLR2	4	rs11938228
		rs3804099
		rs3804100
		rs1585110
CD14	5	rs2569190
LTA	6	rs2229094
TNF	6	rs1799964
		rs1800629
IL6	7	rs1880243
IL6	7	rs1800795
		rs1554606
TLR4	9	rs1927911
		rs2149356
		rs4986791
		rs1554973
MBL2	10	rs1838065
		rs5030737
		rs7096206
		rs930506
IL10RA	11	rs2256111
		rs2229113
		rs11216666
		rs17121510
ACE	17	rs4968779
		rs4293
		rs4341
		rs4351
		rs4267385
		rs8066114
BPI	20	rs1341023
		rs5743507
		rs4358188
IRAK1	X	rs1059703

RESULTS

Population characteristics. A total of 535 newborn infants were enrolled in the study. Demographic and clinical characteristics of the three groups are described in Table 2.

The mean GA and birth weight (BW) for infants with proven sepsis were 27.5 wk and 1169 g, respectively. All infants with proven sepsis were preterm. Most subjects were Caucasian by parental report. Seventy-one infants were classified as late-onset sepsis (>72 h), and eight infants had early-onset sepsis (<72 h). The average age for the first episode of sepsis was 25 d. Forty-one infants had positive blood cultures, 25 infants had positive blood and urine cultures, 12 infants had positive urine cultures, and one infant had a positive CSF culture. Forty-nine percent had a single episode with at least one positive culture, 29% of infants had two episodes, and 22% had three or more episodes during their hospital stay. These episodes were separated in time by at least 3 d, and infants started a new course of antibiotics for each episode. The predominant presenting clinical features of infection were increased apnea episodes (42%), increased oxy-

Table 2. *Demographic and clinical characteristics of the study groups*

Characteristic	Sepsis ($n = 79$)	Suspected ($n = 254$)	Control ($n = 202$)	p*
Gestational age (wk) (min, max)	27.5 (23, 36)	31.7 (23, 36)	33.6 (27, 36)	<0.0001
Birth weight (g) (min, max)	1169 (328, 3440)	1858 (376, 4400)	2120 (606, 4026)	<0.0001
Gender				
Male	59†	129	111	0.0009
Female	20	125	91	
Race				
Caucasian	58	205	176†	0.001
African-American	10†	17	4	
Others (Asian)	5	8	9	
Not reported	6	24	13	
Ethnic status				
Non-Hispanic or Latino	63	185	143	0.92
Hispanic or Latino	7	17	13	
Not reported	9	52	46	
Type of delivery				
C-section delivery	57†	135	109	0.007
Vaginal delivery	22	119	93	
Rupture of membranes (ROM)				
Spontaneous	30	102	72	0.55
Artificial	36	112	99	
No data	13	40	31	
Duration of ROM				
<6 h	14†	38	31	5E-06
6–24 h	1	36	39†	
25–48 h	0	6	7	
>48 h	13†	40†	10	
No PROM	30	66	49	
No data	21	68	66	
ROM (h) (min, max)	40.7 (0, 408)	83.2 (0, 1488)	31.8 (0, 912)	0.04
Clinical chorioamnionitis				
Yes	10†	21†	4	0.002
No	49	172	143†	
Unknown	8	16	8	
No data	12	45	47	
Ventilation days (min, max)	34.2 (0, 106)	7.7 (0, 104)	1.6 (0, 51)	<0.0001
Supplemental oxygen days (min, max)	72.6 (1, 175)	25.0 (0,258)	8.6 (0, 70)	<0.0001

* p values reported are from ANOVA and χ^2 tests. ANOVA is used for gestational age, birth weight, days on ventilation, days on oxygen. All sepsis groups are significantly distinct from each other using Tukey's adjustment for multiple tests at the $p = 0.05$ level. For duration of ROM, only the suspected sepsis group is significantly distinct from the other sepsis groups using Tukey's adjustment for multiple tests at the $p = 0.05$ level. Other variables used Chi-Square tests to determine significant differences between the frequencies within the three sepsis subgroups.

† The subgroup frequency that is overrepresented.

gen requirement or increased need for assisted ventilation (15%), and gastrointestinal signs (12%). In the infected group, one infant died due to a sepsis-related complication.

We found that BW and GA were strongly associated with risk of sepsis ($p < 0.0001$). The infection rate was inversely related to BW. Similarly, the infection rate was inversely related to GA. Seventy-three percent of the infants were ≤28 wk, 14% of infants between 29 and 32 wk, 13% of infants >32 wk. Many low-birthweight neonates have complex medical problems and prolonged hospitalization. The risk of infection is increased in infants with increasing duration of ventilation. Infants with sepsis had a significantly longer duration of mechanical ventilation than those without sepsis ($p < 0.0001$). BW and GA were inversely correlated with days on oxygen and days on ventilation (DOV) (Table 2).

Pathogen distribution. Gram-positive organisms (60%) were isolated more frequently than Gram-negative organisms (30%) in our population. Coagulase-negative *Staphylococcus* (CONS) (50%) was the most commonly isolated organism from blood cultures, and *Enterococcus* species (25%) from urine cultures. The organisms causing sepsis in this investigation are shown in Table 3.

Maternal history. Mode of delivery, duration of rupture of membranes, and presence of clinical chorioamnionitis were significantly associated with sepsis (Table 2).

Analytic results. TDT analysis of 49 SNPs in 19 genes showed that PLA2G2A (rs1891320), *TLR2*(rs3804099), *TLR5* (rs5744105), and *IL10* (rs1800896) were associated with neonatal sepsis ($p < 0.05$). In the suspected group, *PLA2G2A* (rs1891320) and *IL10RA* (rs11216666) showed p values <0.05, with borderline significance for *MBL2* (rs7096206) and *IL6* (rs1800795) in the same group ($p = 0.05$). Haplotype analysis revealed no significant association with sepsis for the SNPs of *PLA2G2A* gene and *TLR2* ($p > 0.05$). The allele frequencies of the significant polymorphisms were next analyzed among the study groups. Infants with sepsis did not show a significantly different frequency of any allele compared with the control group.

Table 3. *Distribution of the pathogens in 79 infants with sepsis (166 episodes)**

	N
Gram-positive infection	
Coagulase-negative *Staphylococcus*	55
Enterococcus	29
Staphylococcus aureus	11
Beta-hemolytic *Streptococcus*	3
Alpha-hemolytic *Streptococcus*	1
Gram-negative infection	
Klebsiella species	18
Escherichia coli	12
Pseudomonas species	6
Serratia species	2
Citrobacter species	5
Haemophilus influenzae	1
Proteus mirabilis	1
Others†	5
Fungal	
Candida (*albican, rugosa, parapsilios*)	17

* Some patients had multiple episodes.

† Others included *Enterobacter* (*cloacae, aerogenes*) and *Acinetobacter baumanii*.

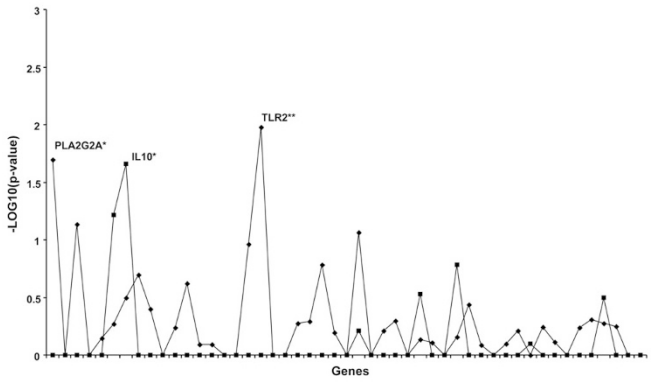

Figure 1. SNPs Associated with Gram-positive (◆) and Gram-negative (■) infections. $**p = 0.01$ and $*p = 0.02$.

We further analyzed each SNP with respect to the type of bacterial infection. Using TDT, we found that allelic variation in *IL10* (rs1800896) was significantly associated with Gram-negative infection ($p = 0.02$), whereas variations in *TLR2* (rs3804099) and *PLA2G2A* (rs1891320) were associated with Gram-positive infection ($p = 0.02$, Fig. 1). Allele frequency of the SNPs associated with Gram-positive and Gram-negative infections did not show any differences with the control group. Haplotype analysis of 2 variations in the *TLR2* gene (rs3804099 and rs3804100) showed significant association with Gram-positive infections ($p = 0.02$). No other haplotype analysis yielded a significant association.

Analysis using spontaneous delivery as the phenotype revealed that the gene *IL8* (rs4073) was significantly associated ($p = 0.004$) with sepsis and this same SNP was also associated with the phenotype prematurity ($p = 0.03$).When regression analysis using GA was done with sepsis as the affection status, none of the aforementioned SNPs showed association. However, variations in the *IL10* gene (rs2222202; $p = 0.03$) and the *IL10RA* gene (rs11216666; $p = 0.02$) showed an association with GA.

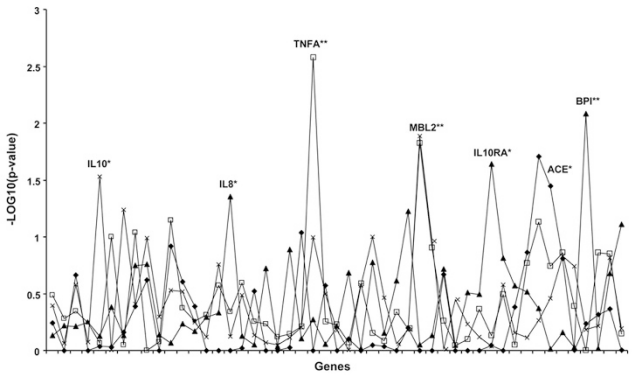

Figure 2. Four phenotypes: a principal component of GA, birth weight, days on oxygen, and DOV for all infants (PrinComponent, □) and only for the 79 sepsis infants (SepsisPrinComp, ◆), GA (GA, ◆) and sepsis adjusted by GA (GA adj, ×). **Three genes have p values <0.01: two for principal component (*TNFA*, $p = 0.003$ and *MBL2*, $p = 0.01$) and one for GA (*BPI*, $p = 0.008$). Sepsis adjusted for GA was also significant for the same SNP in the *MBL2* gene ($p = 0.01$) but was not significant for raw GA. *Four other genes have p values <0.05: *IL10*, $p = 0.03$; *IL8*, $p = 0.04$; *IL10RA*, $p = 0.02$; and ACE has two SNPs with $p = 0.02$ and 0.04.

In a final approach, we used principal components analysis to create another phenotype that was a combination of the four principal predictors of sepsis: GA, BW, the number of days on supplemental oxygen (DOO) and the number of DOV. The eigenvector (or direction of effect) created for all premature infants with data on the four principal predictors of sepsis is −0.525, −0.467, +0.526, and +0.479 for GA, BW, DOO, and DOV, respectively. The eigenvalue (strength of effect) for the first component was 3.04 and represented 76% of the variance. When performing principal components analysis on the 79 infants with sepsis, the eigenvalue was similar (3.21), the variance accounted for was similar (80%), and the eigenvector was similar in magnitude but opposite in direction (+0.521, +0.488, −0.526, and −0.462 for GA, BW, DOO, and DOV, respectively). When the principal component was calculated using all premature infants, TDT analysis identified a SNP in the *MBL2* gene (rs1838065) with a p value of 0.015 and together with rs5030737, a global haplotype p value of 0.05. TDT analysis also identified a SNP in the *TNF* gene (rs1799964) with a p value of 0.002 (T allele associated, 95 informative pedigrees) and a p value of 0.003 for the genotype T/T (Fig. 2). With the principal component calculated just on the infants with sepsis, TDT analysis identified two SNPs in the *ACE* gene (rs4341 and rs4351) with p values of 0.02 and 0.04, respectively, with a haplotype p value of 0.08.

DISCUSSION

Despite the significant burden of sepsis, genetic association studies in newborn infants, especially in VLBW infants, are still limited in comparison with adults (20,24,25). This study used a candidate gene approach and, given the relatively large number of SNPs tested, was viewed as both hypothesis testing and hypothesis generating because the power to detect significant associations after accounting for multiple comparisons ($p < 0.001$ would be the minimal significant p value under stringent testing) was limited by our sample size of just over

500 total cases. Less stringent p values might be explored in the context of generating models to correlate clinical data and specific pathogens interactions. Large-scale population, careful study design, multicenter design, replication studies, repeated confirmation are still needed to have accurate data. In this study, we identified a small number of genetic variations associated with sepsis in preterm infants.

The *PLA2G2A* gene is located on chromosome 1, and the protein it encodes mediates the hydrolysis of phospholipids. Group (G) IIA PLA2 is the most potent among mammalian secreted PLA2(s) against Gram-positive bacteria. Secreted phospholipase A2 enzyme (PLA2) is a mediator connecting innate and adaptive immunity and is up-regulated in infections (26). The sPLA2-IIA is transcriptionally induced by proinflammatory cytokines through the NF-kB pathway. Nevertheless, whether transcriptional synthesis of sPLA2- IIA is regulated by other signaling cascades has not been explored in detail (26). Elsbach and Weiss (27) found that GIIA PLA2, together with the BPI kill Gram-negative bacteria. A number of previous studies have demonstrated *in vitro* and *in vivo* antibacterial properties associated with GIIA PLA2 (28,29). Our study shows that *PLA2G2A* rs1891320 was associated with Gram-positive sepsis. There is no data about the role of this specific SNP in *PLA2G2A* in infections in either neonatal or adult sepsis.

TLRs constitute a family of transmembrane proteins that recognize PAMPs. They play a fundamental role in innate immune responses. TLRs signal via a common pathway that leads to the expression of diverse inflammatory genes. Each TLR elicits specific cellular responses to pathogens using different intracellular adapter proteins. Recent studies have revealed the importance of the subcellular localization of TLRs in pathogen recognition and signaling (30). TLR signaling pathways are negatively regulated by a number of cellular proteins that attenuate inflammation. *TLR2* is predominantly responsible for recognizing Gram-positive cell wall structures (31). Investigators have hypothesized that mutations in TLR2 could be associated with a diminished response to Gram-positive lipoproteins and place individuals at higher risk of Gram-positive infections (21). Sutherland *et al.* (19) reported the association of *TLR2* (rs4696480, T16933A) with the development of sepsis in adults. TLR5, which recognizes bacterial flagellin in Gram-positive and Gram-negative bacteria, plays an important role in mediating responses to this bacterial antigen through activating NF-kB and the release of proinflammatory cytokines (32). Studies suggest that TLR5 plays an important role in pulmonary epithelial responses and may increase susceptibility to pneumonia associated with flagellated organisms (33). Analysis of *TLR5* (rs5744105) was significant in both sepsis and control groups. Hawn *et al.* (33) demonstrated that flagellated bacteria, but not nonflagellated bacteria, activate TLR5, indicating that flagellin is a specific ligand for TLR5. In our study, 23% of septic infants were infected with *Escherichia coli* or *Pseudomonas aeruginosa*, which are flagellated. In the control group, *TLR5* had a p value of 0.02. This finding raises the question as to whether TLR5 may play a role in preterm delivery, a pathologic process that is triggered in part by inflammatory pathways.

Our analysis of *IL10* rs1800896 showed borderline significance ($p = 0.05$), whereas variation at position rs1800872 did not show significance. IL-10 is potent anti-inflammatory cytokine and a key regulator of immune response. Overproduction of either proinflammatory mediators or anti-inflammatory cytokines might lead to organ dysfunction and death (34). In sepsis, high IL-10 levels are associated with severity of infection in newborn infants (35,36). The *IL10* gene 5′-flanking sequence has three SNPs upstream from the transcriptional start site, at positions −1082 (G/A: rs1800896), −819 (C/T), and −592 (C/A: rs1800872), that regulate *IL10* expression (13). Because there is complete linkage disequilibrium between the polymorphisms at −592 and −819, the −819 variant was not evaluated. Baier *et al.* (20) found an increased incidence of late-onset sepsis in VLBW infants with the *IL10* rs1800896 AA genotype, while Treszl *et al.* (24) found no association with susceptibility to sepsis in a small cohort of infants. Schaaf *et al.* (37) in a study of septic shock showed that the *IL10* (rs1800896) G allele is associated with increased IL-10 release, which could be a risk factor for septic shock in pneumococcal infection, while the *IL10* (rs1800872) A allele is associated with lower stimulated IL-10 release and increased mortality.

The National Institute of Child Health and Human Development (NICHD) Neonatal Research Network reported that Gram-negative bacteria continue to be the predominant pathogens associated with early-onset sepsis (38), whereas the majority of late-onset infections were caused by Gram-positive bacteria (1). In our study, we found that one *IL10* polymorphism (rs1800896) was associated with Gram-negative infection, whereas *TLR2* rs3804099 was associated with Gram-positive infections. A study done by Lorenz *et al.* (21) demonstrated a link between a *TLR2* rs5743708 polymorphism and severe staphylococcal infections. Sutherland *et al.* (19) examined another *TLR2* SNP, *TLR2* T-16933A (rs4696480), located 5′ of the *TLR2* gene, in a cohort of patients with sepsis and found an association between the A allele and development of sepsis and Gram-positive cultures. Studies of the amino acid TLR2 Arg753Gln variant (rs5743708) (21,39) suggest that it may predispose individuals to certain Gram-positive infections. No data exist regarding the functional relevance of *TLR2* rs3804099. Thus, overall, there is suggestive evidence that genetic variation in *TLR2* might influence risks for sepsis, but future studies will need to clarify this potential relationship to determine whether rs3804099 itself, or another SNP in association with it, play a causal role.

IL10RA rs11216666, *IL6* rs1800795, and *MBL2* rs7096206 were significant in the suspected sepsis group, but not the control group. *IL6* is a proinflammatory cytokine associated with an increased occurrence of shock and death in septic patients. In our study, *IL6* rs1800795 showed borderline significance in the suspected sepsis group. A large meta-analysis (1323 subjects) to determine the association of the *IL6* polymorphisms and the risk of sepsis in VLBW infants concluded that the available data are not consistent with more than a modest association (25).

BPI, found mainly in the azurophilic granules of neutrophils, plays an important role in the defense against Gram-negative infection and in resolution of endotoxin inflammatory reactions (40). Newborn neutrophils are deficient in BPI (5) and plasma levels tend to be lower in preterm infants (cord blood) than in full term infants (41). Our study did not show an association between the *BPI* polymorphism and susceptibility to sepsis. A reported association between a *BPI* polymorphism and sepsis in a pediatric population (42) suggests that examining *BPI* gene polymorphisms on a larger sample population of preterm and full-term infants is indicated.

Because our study sample is defined as premature infants (<37 wk gestation) with sepsis, suspected sepsis or no sepsis, an important question is how to best adjust for GA. The condition of interest is sepsis, and perhaps the principal components method for defining phenotype is more appropriate since it incorporates degree of sepsis and seriousness of affection into the phenotype as demonstrated by the eigenvector values for DOV and days on oxygen. The fact that most genes had some association with the phenotype of choice indicates that the inflammatory pathway may be involved but different underlying aspects of sepsis are being detected with differing methods. The availability of parental samples and the use of the TDT approach enabled us to eliminate or minimize the effect of ancestry on the results. Nevertheless, it remains possible that particular ancestral groups might have a greater or lesser contribution to the role of a specific SNP in sepsis.

In conclusion, this study of sepsis carried out an analysis of candidate genes in pathways regulating the immune response in a neonatal population. Our data demonstrated uncorrected associations between *PLA2G2A* (rs1891320), *TLR2* (rs3804099), *TLR5* (rs5744105) and *IL10* (rs1800896) polymorphisms and sepsis. Our study is a step in the path of identification of host susceptibility to sepsis and will now require larger and more detailed gene/SNP combinations to be studied. Identification of relevant genetics risk factors can pave the way to a better understanding of pathophysiology and suggest new avenues for treatment and prevention.

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