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The Impact of Environmental and Genetic Factors on Neonatal Late-Onset Sepsis

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Abstract

Objective—To assess the genetic contribution to late-onset sepsis in twins in the newborn intensive care unit (NICU).

Study design—A retrospective cohort analysis of twins born from 1994 to 2009 was performed on data collected from the NICUs at Yale University and the University of Connecticut. Sepsis concordance rates were compared between monozygotic and dizygotic twins. Mixed effects logistic regression (MELR) analysis was performed to determine the impact of selected non-genetic factors on late-onset sepsis. The influence of additive genetic and common and residual environmental effects were analyzed and quantified.

Results—170 monozygotic and 665 dizygotic twin pairs were analyzed and sepsis identified in 8.9%. Mean gestational age and birth weight of the cohort was 31.1 weeks and 1637 grams, respectively. MELR determined birth weight (regression coefficient=−0.001; 95% CI: −0.003–0.000; p=0.028), respiratory distress syndrome (regression coefficient=1.769; 95% CI: 0.943–2.596; p<0.001) and duration of total parenteral nutrition (regression coefficient=0.041; 95% CI: 0.017–0.064; p<0.001) as significant non-genetic factors. Further analysis determined 49.0% (p=0.002) of the variance in liability to late-onset sepsis was due to genetic factors alone, and 51.0% (p=0.001) the result of residual environmental factors.

Conclusions—Our data support significant genetic susceptibility to late-onset sepsis in the NICU population.

Keywords

premature newborn; infection; twins

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Bloodstream infections (BSI) are a relatively common problem in the newborn intensive care unit (NICU) population, particularly in premature neonates.[1–4] Late-onset sepsis (BSI occurring at >72 hours of life) comprises the majority of episodes in this population, with a high rate of associated morbidity and mortality, longer hospital stay and increased costs.[1–10]

Traditionally, neonatologists attribute the high prevalence of late-onset sepsis in the NICU population to a combination of environmental and host factors including, but not limited to, the immature neonatal immune system, a compromised skin barrier, the need for invasive procedures, the prolonged use of invasive life-support apparatus such as endotracheal tubes and central venous catheters, and prolonged hospital stay.[9,11] There is significant individual variability amongst the NICU population with respect to the susceptibility, response to, and outcome associated with late-onset sepsis that may not be explained by these factors alone.[2,11] We hypothesized that, in addition to environmental effects, genetic factors play a major role in predisposing neonates toward developing late-onset sepsis. Using data from a large cohort of monozygotic (MZ) and dizygotic (DZ) twins, we analyzed and quantified the genetic and environmental contributions to late-onset sepsis.

METHODS

Data on all twin pairs born from January 1, 1994 to December 31, 2009 were collected from two medical centers: the University of Connecticut and Yale University. The Institutional Review Boards of each participating center approved the contribution of data to this study.

The zygosity of each twin pair was determined by histopathologic examination of the placenta with additional confirmation using sex concordance or discordance. Late-onset sepsis was specifically chosen as the outcome of interest because, in our (and in most) NICUs, it describes the vast majority of BSI.[1–4] Late-onset sepsis was defined as a blood culture obtained at >72 hours of life that yielded a traditional neonatal pathogen (*e.g. Escherichia coli*) or a commensal species (*e.g. Staphylococcus epidermidis*).[4] Blood cultures that yielded common skin flora such as coagulase-negative staphylococci, which comprised the majority of commensal species-related BSI, were reviewed using specific inclusion criteria from the Centers for Disease Control and Prevention.[12] Although this definition has recently been modified[13], the previous definition[12] was utilized to maintain consistency throughout the study period. Because late-onset sepsis was analyzed as a dichotomous outcome, if an infant had multiple episodes only the first was included.

Respiratory distress syndrome (RDS) was defined as the presence of respiratory distress with an oxygen requirement in the first six hours of life, accompanied by a characteristic chest radiograph. Duration of mechanical ventilation (VENT) was defined as the total number of days that an infant required invasive (*i.e.* via an endotracheal tube) positive pressure ventilation while in the NICU. Positive pressure ventilation included high frequency ventilation and/or intermittent mandatory ventilation. Duration of total parental nutrition (TPN) was defined as the total number of days that the infant required intravenous nutrition while in the NICU. Treating institutions (INST) were the two medical centers where the data were collected: the University of Connecticut and Yale University.

Statistical analyses

Demographic data were analyzed using Student t test, Wilcoxon rank sum test, or chi-square analysis where appropriate.

Chi-square analyses of the zygosity data were performed to compare sepsis concordance rates between MZ and DZ twins. The observed numbers of twin pairs with both infants

affected, with only one infant affected, and with neither infant affected were determined for MZ and DZ groups. These observed numbers formed a 2×3 contingency table and the analog expected numbers of twin pairs were calculated from the corresponding marginal totals. The observed to expected distributions of concordance were compared using chi-square analysis.

Mixed effect logistic regression (MELR) analysis was next performed to identify the impact of selected putative risk factors on sepsis. The covariates utilized in the model included birth sequence, male sex, gestational age (GA), birth weight (BW), RDS, VENT, TPN and INST. The status of the outcomes from twin pairs was treated as a correlated event. A MELR model was fitted to assess the relationship between the covariates listed and the outcome of interest (sepsis), and to incorporate the correlation between twin pairs.

The A (additive genetic) C (common environment) E (unique environment) model described in Feng et al.14 was then used to estimate the variance in liability for sepsis. This mixed effects probit model included covariate effects, an additive genetic effect, a common environmental effect shared by a twin pair (no matter which zygosity it has), and a residual environmental effect. Unique “environmental” effects specific to the NICU population included non-genetic risk factors for late-onset sepsis, such as birth weight, invasive mechanical ventilation, and TPN use. The additive genetic effect, the common environmental effect, and the residual environmental effect were assumed to be independent and normally distributed. Because MZ twins are genetically identical, their additive genetic effects are equal. For DZ twins, the covariance of the additive genetic effects is half that for MZ twins.15 The covariates adjusted in the ACE model included all significant covariates utilized in the MELR analysis. Genetic heritability could then be estimated using the ratio of estimated genetic variance and the total variance of the trait. To confirm the results of the ACE model, we fitted the AE model (without the shared environmental factors) to compare the genetic effects and the residual environmental effects.

An empirical power calculation of the genetic effect analysis was performed to assess the reliability of the model and results. We randomly simulated 100 datasets with the same sample size as our collected data, using the estimates for covariate effects, genetic effects, and environmental effects obtained from the above AE model. Then we used the AE model again to fit each simulated dataset and recorded whether the genetic effect can be significantly identified. An empirical power is the percentage of the times of significant identifications out of these 100 inferences.

Statistical analyses were performed using SAS 9.1 (PROC NLMIXED). A p-value of less than 0.05 was considered statistically significant.

RESULTS

Late-onset sepsis was diagnosed in 149 of 1670 (8.9%) infants from our cohort, which represented 59 of 800 (7.4%) infants from the University of Connecticut and 90 of 870 (10.3%) from Yale University. The incidence of sepsis was determined to be inversely proportional to BW. In the infants with BW < 1000g, 84 of 296 (28.4%) were diagnosed with late-onset sepsis, as compared with 47 of 362 (13.0%) in those with BW 1000 –1499g, and 12 of 540 (2.2%) in infants with BW 1500 –1999g. We also noted that only 6 of 469 (1.3%) infants were diagnosed in the subpopulation with BW ≥ 2000g.

There were 139 (93.3%) episodes of monomicrobial and 10 (6.7%) episodes of polymicrobial late-onset sepsis identified in our cohort. Coagulase-negative staphylococci were the most common organisms isolated (63 of 149 episodes; 42.2%), followed by

Staphylococcus aureus (18 of 149 cases; 12.1%), *Enterococcus* species (13 of 149; 8.7%) and *Klebsiella pneumoniae* (8 of 149; 5.4%).

Zygosity data comprised of 170 MZ and 665 DZ twin pairs were utilized for analysis. The 835 twin pairs had a mean GA and BW of 31.1 weeks and 1637 grams, respectively. Despite a discrepancy in the overall number of twin pairs in each group, no statistically significant differences were observed between MZ and DZ twins with respect to GA, sex, 5-minute Apgar score, the incidence of RDS, duration of mechanical ventilation, duration of TPN, and length of hospital stay (Table I). However, significant difference was found between MZ and DZ twins with respect to BW (Table I).

We initially performed an unadjusted concordance analysis to identify if a genetic effect existed or not for sepsis in our newborn population. The analysis did not reveal a significant difference of concordance distributions between MZ and DZ twin pairs. Table II shows that the concordance rate of the MZ twins was not significantly higher than that of the DZ twins ($p=0.183$).

Next, a MELR analysis was performed using late-onset sepsis as the dependent variable in an attempt to identify significant covariates in our cohort that may have contributed to the outcome of interest. The analysis determined BW, RDS and TPN to be significant predictors for late-onset sepsis (Table III). Excluding cases of polymicrobial sepsis, the significant variables identified by MELR became RDS, VENT and TPN (Table IV; available at www.jpeds.com).

Once significant non-genetic cofactors for sepsis were identified by the MELR analysis, an ACE model which included BW, RDS and TPN as adjusted covariates was employed to estimate the genetic susceptibility to late-onset sepsis. It was determined that 49.8% of the variance in liability to sepsis was the result of genetic factors alone, and 50.2% of the variance in liability to sepsis was the result of residual environmental factors. We also observed that shared environmental factors did not contribute to the variance in liability to sepsis. Therefore we fitted an AE model (without the shared environmental factors) to compare the genetic effects and the residual environmental effects. The AE model determined that 49.0% ($p=0.002$) of the variance in liability to sepsis was the result of genetic factors alone, and 51.0% ($p=0.001$) of the variance in liability to sepsis was the result of residual environmental factors (Table V). Excluding cases of polymicrobial sepsis, using the AE model with the three covariates identified above by MELR, we could still identify significant genetic susceptibility (53.8%, p-value=0.001; Table VI; available at www.jpeds.com). This confirmed the results of the ACE model that about a half of the variance in liability to late-onset sepsis was the result of genetic factors, and the other half was due to residual environmental factors.

The empirical power was calculated to assess the reliability of the model and results. It showed that as 49.0% of the variance in liability to sepsis was according to genetic factors, this genetic effect could be significantly identified 77 times out of 100 replications, based on the sample size of our collected data. This revealed that our sample size was reasonable to detect the degree of genetic effect that we found.

DISCUSSION

We assessed and quantified the influence of additive genetic, common environmental, and residual environmental effects on the host susceptibility to late-onset sepsis and revealed residual (unshared) environmental and genetic factors as nearly equal contributors to the disease process. Lavoie et al assessed the genetic contribution to sepsis in the NICU population via analysis of a cohort of 158 premature twin pairs.[16] As a secondary outcome

of an investigation designed to assess the genetic contribution to bronchopulmonary dysplasia, the authors reported a negligible genetic influence on the susceptibility to bacterial infection (defined as the number of positive blood cultures) in their cohort. In a larger cohort of 835 twin pairs, employing criteria specific for BSI, and utilizing a statistical model which included known potential confounding variables for late-onset sepsis, we were able, for the first time, to quantify both the genetic and environmental effects on this disease process.

In our analysis, the environmental influences on late-onset sepsis were separated into shared and unshared (residual) components. Shared environmental effects (*e.g. in utero*; maternal and intrapartum variables) did not have a significant influence on the susceptibility to late-onset sepsis. These results support the belief that these variables are generally considered risk factors for early (≤ 72 hours of life), but not late-onset sepsis. Residual environmental effects (*e.g.* co-morbidities and other variables related to the hospital course) were determined to account for a significant component of the variance in liability to late-onset sepsis. This finding supports the previous understanding of late-onset sepsis as a hospital-acquired disease process in a highly vulnerable patient population. The major independent risk factors previously identified for late-onset sepsis include, but are not limited to, the use and duration of use of central venous catheters, TPN, and mechanical ventilation.[2,17,18] Despite knowledge of these and other risk factors, there is significant variability in the rates of late-onset sepsis, even in NICUs that provide comparable levels of care.[2] The National Institute of Child Health and Human Development Neonatal Research Network, comprised of 16 tertiary NICUs, reported center-to-center rates of late-onset sepsis ranging from 10.6% to 31.7%.[2] Similar patient-to-patient variability is observed within individual NICUs suggesting other factors may play a role.

A genetic component to infection has long been hypothesized in both the adult and pediatric patient populations and supported through various investigative approaches. In a landmark epidemiologic investigation, Sorensen et al[19], utilizing the Danish Adoption Registry, determined that an adoptee had a near six-fold increased risk of an infectious disease-related death if their biologic parent had died from an infection prior to age 50. No such increased risk existed if a similar death occurred in the adoptive parent. The authors concluded that untimely deaths of an infectious etiology carry a strong genetic component.[19] The genetic contribution to specific infectious diseases has also been investigated in MZ and DZ twins. Higher rates of disease concordance among MZ twin pairs have suggested a genetic component to chronic infectious diseases such as tuberculosis20, poliomyelitis,21 and hepatitis B.22,[23] These epidemiologic investigations have served as the foundation for molecular studies aimed at determining the specific role of single nucleotide polymorphisms (SNP) in the variability in onset and response to infection. Some of these investigations have yielded positive associations between polymorphisms in the genes which encode pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α[24–]26, TNF-β27, interleukin-628, and angiotensin I converting enzyme[29] and an increase in the severity in the response to, and outcome from, sepsis.[30] Others have linked genetic polymorphisms in the anti-inflammatory cytokine interleukin receptor antagonist with an increase in the susceptibility to infection.[31,32]

Although these data would seem to support a genetic contribution to sepsis, the majority of investigations to date have been conducted in subjects from demographically, geographically, and developmentally diverse patient populations. In fact, the majority of epidemiologic data on the subject comes from cohorts of adults and older children. There are similar pitfalls inherent to SNP association studies. Single-gene associations detected in underpowered investigations conducted in relatively homogeneous patient populations may be unreliable.[33,34] Furthermore, candidate genes associations identified in adults and older

children may not correspond with similar findings in premature neonates due to developmental regulations in gene expression.[29,35–37] Genetic polymorphism for angiotensin I converting enzyme, for example, have been associated with a more severe outcome in older children with sepsis[29], but no such association was found to exist in premature neonates.[36,37] In order to identify genetic factors specific to this disease process and population, future investigations will likely need to focus on a developmentally appropriate population.

There are several limitations to our data that may limit interpretation. One potential pitfall relates to our definition of BSI, particularly in the setting of commensal species related sepsis. Despite efforts to minimize inclusion of false-positive blood cultures into our data, it is possible that some cases of BSI included were not true cases of late-onset sepsis, but were instead contaminants. However, all episodes of commensal species related sepsis met strict inclusion criteria that incorporated clinical signs and symptoms of infection, a positive blood culture or blood cultures, and treatment with appropriate antimicrobial agents. Additionally, excluding the episodes of polymicrobial sepsis did not significantly impact our results. It is also possible that the genetic effect on late-onset sepsis may be species specific and by grouping all late-onset sepsis together, we may have overestimated the genetic contribution in some cases and underestimated it in others. Unfortunately, this investigation was not powered for additional, species-specific subgroup analyses. In addition, our cohort was restricted to twin pairs with available zygosity information and therefore represented a limited, but still a fairly substantial, number of infants. The zygosity was determined using placental histopathology and sex, as we did not have DNA for confirmation. A monochorionic placenta was assumed to represent MZ twins. About 9% of similar sex dichorionic placentas are MZ.[38] Our conclusions were not affected when adjustments were made for such worst-case scenarios. Lastly, the covariates collected and analyzed in our dataset did not include all potential contributing factors related to late-onset sepsis. Complete data were not available, for example, on central versus peripheral venous catheter use, although we were able to account for the effects of BW, mechanical ventilation, and the use of TPN (a marker for the need for intravascular access).

Our data indicate that genetic and environmental factors play a near equal role in this disease process. At the present time, strategies to control late-onset sepsis should continue to focus on limiting the risk of environmental factors such as central lines. In the future, more sophisticated knowledge of, and approaches to, the human genome may further improve our ability to identify and properly manage those at risk.

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ABBREVIATIONS

BSI	bloodstream infections
NICU	newborn intensive care unit
VLBW	very low birth weight
GA	gestational age
BW	birth weight
MZ	monozygotic

DZ	dizygotic
RDS	respiratory distress syndrome
VENT	duration of (invasive) mechanical ventilation
TPN	duration of total parenteral nutrition
INST	treating institutions
MELR	mixed effects logistic regression
SNP	single nucleotide polymorphisms
TNF	tumor necrosis factor

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Table I

Comparison of MZ and DZ twin pairs

Variable	MZ (N=170)	DZ (N=665)	p
GA (weeks)	31.0 ± 3.1*	31.1 ± 3.3	0.883
BW (grams)	1579 ± 572	1652 ± 688	0.045
Male gender	180 (52.9)**	691 (52.0)	0.812
5-minute Apgar score	9†	9	0.091
RDS	148 (44.4)	534 (40.8)	0.252
VENT (days)	6.9 ± 15.5	7.5 ± 20.1	0.563
TPN (days)	9.9 ± 17.3	10.2 ± 18.4	0.811
Length of stay (days)	39.6 ± 33.9	36.9 ± 36.2	0.201
Late-onset sepsis	38 (11.2)	111 (8.3)	0.127

*Mean ± SD

**N (%)

†Median

GA: gestational age; BW: birth weight; RDS: respiratory distress syndrome; VENT: duration of (invasive) mechanical ventilation; TPN: duration of total parenteral nutrition

Table II

Zygosity analysis for late-onset sepsis

Twin pairs	Both have sepsis	One has sepsis	Neither has sepsis	Total	p
MZ	9 (5.3)*	20 (19.7)	141 (145.0)	170	
DZ	17 (20.7)	77 (77.3)	571 (567.0)	665	
Total	26	97	712	835	0.183

* Observed number of twin pairs (expected number of twin pairs)

Table III

Mixed effects logistic regression analysis for late-onset sepsis

Variable	Coefficient	95% CI	p
Birth Sequence	0.134	[−0.387, 0.654]	0.615
Male	0.393	[−0.228, 1.014]	0.214
GA	0.076	[−0.142, 0.293]	0.495
BW	−0.001	[−0.0027, −0.0002]	0.028
RDS	1.769	[0.943, 2.596]	<0.001
VENT	0.016	[−0.004, 0.035]	0.109
TPN	0.041	[0.017, 0.064]	<0.001
INST (Yale)	0.152	[−0.557, 0.861]	0.675

GA: gestational age; BW: birth weight; RDS: respiratory distress syndrome; VENT: duration of (invasive) mechanical ventilation; TPN: duration of total parenteral nutrition; INST: treating institution

Table IV

Mixed effects logistic regression analysis for late-onset sepsis (excluding cases of polymicrobial sepsis)

Variable	Coefficient	95% CI	p
Birth Sequence	0.807	[−0.044, 1.658]	0.063
Male	0.627	[−0.410, 1.664]	0.235
GA	0.317	[−0.090, 0.725]	0.127
BW	−0.002	[−0.0039, 0.0004]	0.103
RDS	2.224	[0.740, 3.709]	0.003
VENT	0.044	[0.012, 0.076]	0.008
TPN	0.103	[0.051, 0.154]	<0.001
INST (Yale)	−0.182	[−1.557, 1.193]	0.795

GA: gestational age; BW: birth weight; RDS: respiratory distress syndrome; VENT: duration of (invasive) mechanical ventilation; TPN: duration of total parenteral nutrition; INST: treating institution

Table V

AE model analysis for late-onset sepsis

Variables/Effects	Estimate	p
BW	−0.001	<0.001
RDS	1.122	<0.001
TPN	0.025	<0.001
Genetic	0.490	0.002
Residual Environmental	0.510	0.001

BW: birth weight; RDS: respiratory distress syndrome; TPN: duration of total parenteral nutrition

Table VI

AE model analysis for late-onset sepsis (excluding cases of polymicrobial sepsis)

Variables/Effects	Estimate	p
RDS	1.235	<0.001
VENT	0.014	0.009
TPN	0.026	<0.001
Genetic	0.538	0.001
Residual Environmental	0.462	0.003

RDS: respiratory distress syndrome; VENT: duration of (invasive) mechanical ventilation; TPN: duration of total parenteral nutrition