Acylcarnitine Profiles Reflect Metabolic Vulnerability for Necrotizing Enterocolitis in Newborns Born Premature

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Objective To evaluate the association between newborn acylcarnitine profiles and the subsequent development of necrotizing enterocolitis (NEC) with the use of routinely collected newborn screening data in infants born preterm.

Study design A retrospective cohort study was conducted with the use of discharge records for infants born preterm admitted to neonatal intensive care units in California from 2005 to 2009 who had linked state newborn screening results. A model-development cohort of 94 110 preterm births from 2005 to 2008 was used to develop a risk-stratification model that was then applied to a validation cohort of 22 992 births from 2009.

Results Fourteen acylcarnitine levels and acylcarnitine ratios were associated with increased risk of developing NEC. Each log unit increase in C5 and free carnitine /(C16 + 18:1) was associated with a 78% and a 76% increased risk for developing NEC, respectively (OR 1.78, 95% CI 1.53-2.02, and OR 1.76, 95% CI 1.51-2.06). Six acylcarnitine levels, along with birth weight and total parenteral nutrition, identified 89.8% of newborns with NEC in the model-development cohort (area under the curve 0.898, 95% CI 0.889-0.907) and 90.8% of the newborns with NEC in the validation cohort (area under the curve 0.908, 95% CI 0.901-0.930).

Conclusions Abnormal fatty acid metabolism was associated with prematurity and the development of NEC. Metabolic profiling through newborn screening may serve as an objective biologic surrogate of risk for the development of disease and thus facilitate disease-prevention strategies. *(J Pediatr 2017;181:80-5).*

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Necrotizing enterocolitis (NEC) is a leading cause of morbidity and mortality among infants born preterm. NEC is an acquired disease of the neonatal period marked by inflammation and necrosis of the gastrointestinal tract. The ambiguity of presenting symptoms of NEC and the low specificity of common diagnostic tests may lead to delayed diagnosis and treatment.[1]

The underlying pathophysiology of NEC appears to be multifactorial, involving a combination of developmental immaturity, variable feeding practices, and bacterial colonization of the gut.[2] Metabolism emerges at the intersection of these predisposing variables as an underexplored feature that likely impacts disease onset. Because no previous studies conclusively have identified high-risk infants based on measurable predisposing biologic features, there has been little progression in the understanding of the inciting pathophysiologic basis for NEC beyond prematurity.[3-5]

Abnormal fatty and organic acid metabolism of prematurity as indicated by acylcarnitine profiles may be implicated in the pathogenesis of NEC. Prematurity-associated disturbances in nutrient metabolism, enteric dysmotility, and gut colonization may result in excess fermentation and the accumulation of organic and short-chain fatty acids that have been shown to contribute to intestinal mucosal injury and necrosis in both human subjects and animal models that closely mimic human NEC.[6-10] We hypothesized that an association between newborn acylcarnitine profiles and the subsequent development of NEC could further refine age- and weight-associated risk in biologic terms.

AUC	Area under the curve
FC	Free carnitine
NEC	Necrotizing enterocolitis

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Methods

To explore the relationship between the metabolism of newborns born premature and NEC, newborn screening results were obtained from 94 110 singleton (<37 completed weeks' gestation) newborns born preterm who had routine newborn screening between 12 hours and 8 days of age through the Genetic Disease Screening Program within the California Department of Public Health between 2005 and 2008 and had linked birth certificate and hospital discharge records. The naïve validation cohort consisted of 22 992 singletons born preterm with births between January 1 and November 30, 2009, who also had newborn screening between 12 hours and 8 days of age and also had linked birth and hospital discharge records. Details regarding the populations from which the model-development and validation cohorts were drawn are included in the **Figure** (available at www.jpeds.com). The study was approved by the Committee for the Protection of Human Subjects within the Health and Human Services Agency of the State of California by waiver of informed consent.

Acylcarnitine Measurements

Acylcarnitine measurements, hours/days after birth at testing, race/ethnicity, and information about whether the infant had been on total parenteral nutrition between birth and the time of testing were obtained from the newborn screening records. Birth certificate and hospital discharge records were linked to newborn screening data through the California Office of Statewide Health Planning and Development to obtain information on total days gestation, birth weight, and diagnosis of NEC (by *International Statistical Classification of Diseases, Ninth Revision*, code 777.5). Details regarding the newborn screening program and testing of acylcarnitine levels have been described in detail.[11,12] All newborns included in the present study had acylcarnitine levels measured in dried blood specimens collected by heel-stick at birth hospitals between 12 hours and 8 days after birth.

Following collection, specimens were sent to a state-approved laboratory for testing with a standardized tandem mass spectrometry assay (MS^2 2000 system; PerkinElmer Life Sciences, Shelton, Connecticut). Specimens were tested with a NeoGram acylcarnitine derivatized reagent kit (PerkinElmer). For all samples, testing was based on the MS^2 system operated in the positive ion mode (source voltage: 5500 V). Acylcarnitine levels were measured by precursor ion scanning with precursors of m/z 85 and quantitated by comparison with stable-isotope internal standards. All information on acylcarnitine levels measured as part of routine newborn screening was included in the analyses. This included values for 20 acylcarnitines (C2, C3, C3DC, C4, C5, C5:1, C5DC, C6, C8, C8:1, C10, C10:1, C12, C14, C14:1, C16, C16:1, C18, C18:1, C18:1OH, and free carnitine [FC]) and 2 acylcarnitine ratios (FC/[C16 + C18:1] and C3/C2).

Model Development and Validation Cohort Analyses

Two analyses were conducted. First, the association between acylcarnitine levels and a subsequent diagnosis of NEC was evaluated in the 2005-2008 model-development cohort. Second, the performance of these acylcarnitine levels and acylcarnitine ratios in identifying infants born preterm at risk for NEC was evaluated in the 2005-2008 model-development cohort and in the 2009 validation cohort. Inclusion in the 2009 cohort was limited to those with a birth before December because of a change in laboratory assay in December 2009.

Analysis of Individual Acylcarnitine Levels

Crude association testing in the model-development cohort included the comparison of newborns born preterm with and without NEC by characteristic and by the log of acylcarnitine level and ratio. The χ^2 test was used to compare groups by race/ethnicity, sex, total parenteral nutrition (yes or no), age in days at acylcarnitine testing, and gestational age (gestational age <32, 32-36 weeks) by birth weight grouping (<1500, 1500-2499, ≥2500 g). Race/ethnicity was derived from the birth certificate record, where the reporting parent selected from a list of predefined categories. The 2-tailed Wilcoxon rank sum test was used for the initial comparison of the distribution of acylcarnitine level and ratios between infants born preterm with and without NEC. Logistic regression was used to calculate ORs and 95% CIs to identify the relationship between a natural log-unit increase in acylcarnitine levels or ratios and the risk of NEC wherein both crude- and characteristic-adjusted risks were evaluated.

Multivariate Analysis of Acylcarnitine Levels

Final model development for combined characteristic and acylcarnitine effects used backward stepwise regression methods in which $P < .10$ was used as the threshold for entering the model and $P < .05$ was used as the threshold for remaining. We evaluated the performance of the final logistic model for NEC prediction in both the model-development and validation cohorts. Receiver operator characteristic curves and associated area under the curve (AUC) statistics were evaluated overall, by day of testing, and by gestational age. All analyses were performed with SAS Software, version 9.3 (SAS Institute, Cary, North Carolina) based on data received by the Genetic Disease Screening Program as of December 31, 2013.

Results

Most newborns in the model-development cohort were Hispanic (50.92%) or non-Hispanic white (27.05%) and had newborn screening obtained between 12 hours and 2 days of life (69.18%). Approximately 1 in 127 infants born preterm ultimately was diagnosed with NEC. Of those who developed NEC, the greatest frequency was seen in newborns with births before 32 completed weeks of gestation with birth weight < 1500 g (**Table I**). Preterm newborns with NEC in the model-development and validation cohorts differed from those without NEC by race/ethnicity, use of total parenteral nutrition at the time of testing, day of life at testing, and by gestational age by birth weight grouping (**Table I**).

Analysis of Individual Acylcarnitines

The distribution of acylcarnitine levels and acylcarnitine ratios in infants born preterm with and without NEC differed across