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Acylcarnitine Profiles Reflect Metabolic Vulnerability for Necrotizing Enterocolitis in Premature Newborns

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Abstract

Objectives—Given the known risk factors for NEC, we hypothesized that metabolic dysfunction reflected in routinely collected newborn screening data would be associated with NEC in an at risk population.

Study Design—We conducted a retrospective cohort study using discharge records for all preterm neonatal intensive care unit admissions in California from 2005 to 2009. Infants with linked state newborn screening results were included. A model-development cohort of 94,110

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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 acylcarnitines and acylcarnitine ratios were associated with increased risk of developing NEC. Each log unit increase in C5 and FC/(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 acylcarnitines, along with birth weight and total parenteral nutrition, were able to identify 89.8% of newborns with NEC in the model-development cohort (AUC=0.898, 95% confidence interval (CI) 0.889 – 0.907) and 90.8% of the newborns with NEC in the validation cohort (AUC=0.908, 95% CI 0.901 – 0.930).

Conclusions—These findings demonstrate that abnormal fatty acid metabolism is 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.

Keywords

metabolism; prematurity; newborn screen; fatty acids

Introduction

Necrotizing enterocolitis (NEC) is a leading cause of morbidity and mortality among preterm infants. 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 lead to delayed diagnosis and inability to initiate targeted therapies.(1)

The underlying pathophysiology of NEC is 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 under-explored feature that likely impacts disease onset. Since no prior studies have conclusively identified high-risk infants based upon measurable predisposing biologic features, there has been little progression in the understanding of the inciting pathophysiologic basis for NEC beyond prematurity.(3-5)

Newborn screening (NBS) reports essential biomarkers that taken together are utilized to identify possible metabolic dysfunction associated with genetic disease. It is now well established that NBS metabolites including amino acids and acylcarnitines vary according to gestational age and birth-weight.(6-10) Gestational age and newborn weight as measures of developmental immaturity have long been used to aid in the determination of risk for acquired diseases of prematurity like NEC. As an alternative, NBS panels may be used to identify a predisposing metabolic phenotype that is associated with an acquired disease of prematurity such as NEC.

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 can 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.(11-15) 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.

Patients and Methods

Patient populations

To explore the relationship between premature newborn metabolism and NEC, we used newborn screening results from more than 100,000 singleton preterm newborns born in California between 2005 and 2009. The model-development cohort consisted of singleton preterm (< 37 completed weeks gestation) newborns (n = 94,110). All subjects were born in California between 2005 and 2008, had routine newborn screening through the Genetic Disease Screening Program within the California Department of Public Health with a serum draw between 12 hours to 8 days of birth, and had linked birth certificate and hospital discharge records. The naïve validation cohort consisted of 22,992 preterm singletons with births between January 1 and November 30, 2009 who also had newborn screening based on serum collected between 12 hours and 8 days of birth 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 Figure 1(online only).

Acylcarnitine measurements

We obtained 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 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 from which we obtained information on total days gestation, birth weight, and diagnosis of NEC (by ICD-9 code 777.5). Details regarding the newborn screening program and testing of acylcarnitines have been described in detail elsewhere.(16, 17) In brief, all newborns included in the present study had acylcarnitines 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 using a standardized tandem mass spectrometry assay (MS^2 2000 system (PerkinElmer Life Sciences, Shelton, CT)). Specimens were tested using 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). Acylcarnitines were measured by precursor ion scanning using precursors of *m/z* 85, and quantitated by comparison to stable-isotope internal standards. All information on acylcarnitines measured as part of routine newborn screening was included in analyses. This included values for twenty 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 two acylcarnitine ratios (FC/(C16+C18:1) and C3/C2).

Model Development and Validation cohort analyses

We performed two distinct phases of analysis. First, we evaluated whether there was an association between acylcarnitines and a subsequent diagnosis of NEC in the 2005 - 2008 model-development cohort. Second, we evaluated the performance of these acylcarnitines and acylcarnitine ratios in identifying preterm infants at risk for NEC in the 2005 to 2008 model-development cohort and in the 2009 validation cohort (wherein inclusion in the 2009 cohort was limited to those with a birth before December due to a change in lab assay in December, 2009).

Analysis of individual acylcarnitines

Crude association testing in the model-development cohort included comparing preterm newborns with and without NEC by characteristic and by the log of acylcarnitine level and ratio. The chi square test was used to compare groups on race/ethnicity, sex, total parenteral nutrition (yes or no), age in days at acylcarnitine testing, gestational age (gestational age <32, 32-36 wks) by birth weight grouping (< 1500, 1500-2499, ≥ 2500 grams). Race/ethnicity was derived from the birth certificate record where the reporting parent selected from a list of predefined categories. We used the two-tailed Wilcoxon Rank Sum Test for initial comparison of the distribution of acylcarnitine level and ratios between preterm infants with and without NEC. We then performed logistic regression to calculate odds ratios and 95% confidence intervals 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 acylcarnitines

Final model development for combined characteristic and acylcarnitine effects utilized backward stepwise regression methods where $p < .10$ was used as the threshold for entering the model and $p < .05$ was used as the threshold for remaining. We evaluated 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 statistics were evaluated overall, by day of testing, and by gestational age.

Statistical software and study approval

All analyses were performed using Statistical Analysis Software (SAS) version 9.3 (Cary, NC) based on data received by the Genetic Disease Screening Program as of December 31, 2013. This 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.

Results

Patient characteristics

Most newborns in the training 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 preterm infants was ultimately diagnosed with NEC. Of

those that developed NEC, the highest frequency was seen in newborns with births before 32 completed weeks of gestation with birthweight < 1500 grams (Table 1). 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 1).

Analysis of individual acylcarnitines

The distribution of acylcarnitines and acylcarnitine ratios in preterm infants with and without NEC was different across all measures except for log C2, log C6 and log C18:1OH (Table 2, online). Fourteen of the 23 acylcarnitine measures were associated with per log unit increases in NEC risk after adjustment for race/ethnicity, use of total parenteral nutrition, days at test (by grouping), and gestational age by birth weight grouping (Table 3, online). Each log unit increase in C5 was associated with a 78% increased risk for NEC after adjustment (odds ratio 1.78, 95% confidence interval 1.53 – 2.02). Each log unit increase in FC/(C16+18:1) was associated with a 76% increase in risk for NEC after adjustment (odds ratio 1.76, 95% confidence interval 1.51 – 2.06) (Table 3, online).

Combined Risk of NEC Acylcarnitines model

When patient characteristics, acylcarnitines, and acylcarnitine ratios were evaluated together, five acylcarnitines (log C5, log C5:1, log C8:1, log C12, log C14:1), one acylcarnitine ratio (log FC/(C16+C18:1)), gestational age by birth weight grouping, and use of total parenteral nutrition were found to significantly associate with NEC at $p < .05$ (Table 4). This combination of factors was able to correctly group preterm infants with and without NEC 89.8% of the time (area under the curve=0.8983, 95% confidence interval 0.8895 – 0.9072) in the model-development cohort and 90.8% of the time (area under the curve=0.9078, 95% confidence interval 0.8903 – 0.9253) in the validation cohort (Table 5).

Acylcarnitines, Prematurity and Biologic Vulnerability

Model performance was the best among those with newborn screening obtained between 12 hours and 2 days of life (area under the curve=0.9339, 95% confidence interval 0.9236 – 0.9440 in the model development cohort and area under the curve=0.9518, 95% confidence interval 0.9380 – 0.9655 in the validation cohort) (Table 5). When characteristics and acylcarnitines were considered in isolation, both sets of factors were associated with AUCs > 85% overall, > 70% in newborns with gestational ages < 32 weeks, and > 80% in newborns with gestational ages between 32 and 36 weeks in both the model development and validation cohorts (Table 6). In both the development and validation cohorts, increased AUCs were observed when characteristics and acylcarnitines were considered together, although in general, increases were modest.

Discussion

Herein we provide the first report of an observed association between fatty acid metabolism (acylcarnitine profiles) and NEC in premature newborns. These observations demonstrate that metabolic profiles obtained at birth reflect biologic vulnerability prior to any alteration by clinical care. These data provide important pathophysiologic insights into newborn

systemic metabolic function that predisposes the vulnerable premature to acquired disease like NEC and could therefore support the development and testing of prevention strategies. This potential novel application of newborn screening data demonstrates a widely available vehicle for further development as a risk stratification mechanism.

Prior biomarker studies have attempted to identify high-risk populations early in the course of disease and to differentiate NEC from other neonatal inflammatory conditions.(18-27) These reports have largely focused on inflammatory pathways and have therefore used combinations of non-specific markers that have failed to identify high-risk infants in a timeframe that would allow implementation of disease prevention strategies. Our results introduce the concept of utilizing NBS at birth to identify metabolic dysfunction and the link to the acquired disease of prematurity NEC. Accordingly, acylcarnitine levels measured within the first several days of life may provide an opportunity for early risk stratification and a method for testing various metabolism based prevention strategies including probiotics or modified feeding protocols that have shown some promise in prior clinical studies.(28, 29)

Metabolism and NEC

The underlying pathophysiology of NEC remains incompletely understood and is likely multifactorial. The combination of prematurity, variable feeding practices and bacterial colonization are consistently implicated as the major predisposing factors.(1, 2) Although the proximal event leading to mucosal injury is not well defined, it is conceivable that premature newborns are predisposed to NEC as a result of compromised fatty acid metabolism. Since acylcarnitines are derived from the metabolism of fatty and organic acids, it is plausible that abnormal systemic fatty acid oxidation predisposes to gut specific toxicity following the introduction of a metabolic challenge as occurs with enteral feedings. It has been previously reported in animal models of prematurity that exposure of the intestinal mucosa to fatty acid derivatives causes mucosal necrosis.(12, 13, 30)

NEC is most commonly diagnosed after the initiation of enteral feeding and may be related to both the timing (early or late) of initiation of enteral feedings and rate of feeding advancement, thus implying that increased exposure of the premature gastrointestinal lumen to gut fermentation products including fatty and organic acids produces NEC inciting injury.(31-33) It is intriguing that total parenteral nutrition appears to be a risk factor for the development of NEC both in this study and others.(34) It is unclear whether total parenteral nutrition is exacerbating metabolic dysfunction or is simply a surrogate for sicker preterm infants who begin enteral feeding in a delayed manner. Importantly, the acylcarnitine-NEC association described in this study was most accurate for infants who underwent screening within the first 48 hours of life perhaps suggesting that metabolic profiling may reflect development dependent metabolic dysfunction. Additional studies evaluating the gastrointestinal toxicity of dysfunctional metabolism involving fatty acid oxidation in both laboratory and clinical studies of NEC is warranted to confirm these speculations.

Clinical Utility and Insights

Despite good overall model sensitivity, the utility of the current acylcarnitine-based model as a clinical tool requires additional consideration. The addition of other routinely measured metabolic parameters (*e.g.* amino acids) and serial testing may both improve on the statistical performance and positive predictive value of metabolic profiling as a clinical prediction tool as well as account for clinical care confounding and influence on metabolic risk longitudinally. A reasonable objective may be to utilize the present metabolic model to facilitate the development of novel management and prevention strategies based upon metabolic profiling. The potential risks and benefits of promising NEC prevention strategies (including monitored feeding protocols and/or probiotics) are subject to ongoing study.(32, 35) However, given the substantial NEC related mortality as well as the possibility of significant life-long gastrointestinal and neurologic impairment in survivors, the potential benefit from the prevention of any case of NEC should be viewed as highly significant relative to the potential for harm of perceived low risk interventions.

Strengths and Limitations

The strengths of the present study include the use of population-based metabolic screening data linked to a comprehensive neonatal outcomes database. This combination has expanded the novel application of linking newborn screening results to acquired newborn disease.(36, 37) Our study population included preterm infants from across the broad geographic and socioeconomic regions of California and the results remained robust while controlling for multiple patient demographic factors. In light of these strengths it should also be recognized that there are important limitations to the present study. The use of a population-based dataset meant that we relied exclusively on hospital discharge records for NEC diagnosis and were therefore limited in our ability to stratify results by severity of disease (progressive and non-progressive NEC) through the examination of clinical records. Further, since the validation cohort was also derived from California births (albeit in a different year) may have led to some over fitting of the model. These issues point to the importance of testing the relationships observed in the current study in other populations where tighter phenotypic description is possible. Accordingly, subsequent efforts will benefit from a focus on targeted age and size cohorts (*e.g.* <32, or 32-26 weeks) exclusively given the discussed etiologic and clinical implications.

Summary

The observed association between acylcarnitine profile and NEC offers the potential for early identification of high-risk newborns based upon metabolism utilizing an available testing platform and represents an important first step towards directing preventive measures and developing improved therapeutic strategies. The present findings suggest that NEC may be the manifestation of a predisposing systemic metabolic dysfunction thus providing new insights to the pathophysiology of NEC.

Conclusions

There is an association between abnormal acylcarnitine profiles measured during newborn screening in premature infants and risk for NEC. Replication and external validation of the findings may lead to the development of novel prevention strategies.

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Abbreviations

NEC	necrotizing enterocolitis
NBS	newborn screening
AUC	area under the curve

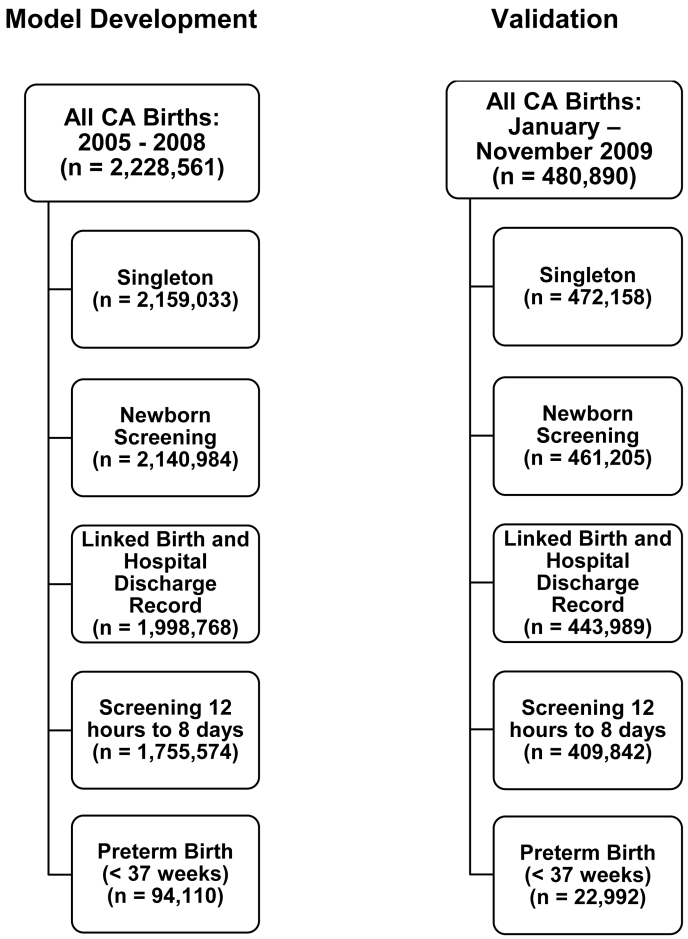

Figure 1.

Model Development and Validation cohort exclusions.

Legend: Details regarding populations from which the model-development and validation cohorts were drawn.

Table 1

Descriptive characteristics – Training and testing cohorts: Preterm births with and without necrotizing enterocolitis.

	Model Development				Validation			
	All n =	**No NEC n (%)**	**NEC n (%)**	**X^2**	**All n =**	**No NEC n (%)**	**NEC n (%)**	**X^2**
Sample	94,110	93,366	744		22,992	22,876 (99.50)	116 (0.50)	
Race/Ethnicity								
White	25,460 (27.05)	25,270 (27.07)	190 (25.54)		6,021 (26.19)	5,992 (26.19)	29 (25.00)	
Hispanic	47,923 (50.92)	47,579 (50.96)	344 (46.24)		11,663 (50.73)	11,608 (50.74)	55 (47.41)	
Asian	8,509 (9.04)	8,436 (9.04)	73 (9.81)		2,213 (9.63)	2,203 (9.58)	10 (8.62)	
Black	7,547 (8.02)	7,448 (7.98)	99 (13.31)		1,883 (8.19)	1,869 (8.17)	14 (12.87)	
Native American	260 (0.28)	257 (0.28)	3 (0.40)		53 (0.23)	52 (9.63)	1 (0.86)	
“Other Race”	3,116 (3.31)	3,091 (3.31)	25 (3.36)		818 (3.56)	815 (3.56)	3 (2.59)	
Unknown	1,295 (1.38)	1,285 (1.38)	10 (1.34)	30.95[b]	341 (1.48)	337 (1.47)	4 (3.45)	7.94
Sex[a]								
Male	42,678 (45.35)	42,346 (45.35)	332 (44.62)		12,571 (54.68)	12,506 (54.67)	65 (56.03)	
Female	51,396 (54.61)	50,984 (54.61)	412 (55.38)	0.45	10,412 (45.29)	10,361 (45.29)	51 (43.97)	0.13
Total Parenteral Nutrition								
Yes	13,928 (14.80)	13,506 (14.47)	422 (56.72)		5,800 (25.23)	5,701 (24.92)	99 (85.34)	
No	80,182 (85.20)	79,860 (85.53)	322 (43.28)	1045.16[b]	17,192 (74.77)	17,175 (75.08)	17 (14.66)	223.39[b]
Hours/Days at Testing								
12 hours – 2 days	65,108 (69.18)	64,786 (69.39)	322 (43.28)		16,158 (70.28)	16,112 (70.43)	46 (39.66)	
3 – 4 days	19,310 (20.52)	19,066 (20.42)	244 (32.80)		4,700 (20.44)	4,660 (20.77)	40 (34.48)	
5 – 6 days	8,669 (9.21)	8,517 (9.12)	152 (20.43)		1,961 (8.53)	1,936 (8.46)	25 (21.55)	
7 – 8 days	1,023 (1.09)	997 (1.07)	26 (3.49)	270.27[b]	173 (0.75)	168 (0.73)	5 (4.31)	69.60[b]
Gestational Age								
< 32 Weeks	17,550 (18.65)	16,982 (18.19)	568 (76.34)		4,269 (18.57)	4,180 (18.27)	89 (76.72)	260.80 [b]
32 – 36 Weeks	76,560 (81.35)	76,384 (81.81)	176 (23.66)	1645.51[b]	18,723 (81.43)	18,696 (81.73)	27 (23.28)	
Gestational Age by Birth Weight								

	Model Development				Validation			
	All n =	No NEC n (%)	NEC n (%)	X^2	All n =	No NEC n (%)	NEC n (%)	X^2
< 32 Weeks								
< 1500 grams	7,828 (8.32)	7,363 (7.89)	465 (62.50)		2,153 (9.36)	2,072 (9.06)	81 (69.83)	
1500 – 2499 grams	4,285 (4.55)	4,189 (4.49)	96 (12.90)		1,057 (4.69)	1,049 (4.59)	8 (6.90)	
≥ 2500 grams	5,437 (5.78)	5,430 (5.82)	7 (0.94)		1,059 (4.61)	1,059 (4.63)	--	
32 – 36 Weeks								
< 1500 grams	1,817 (1.93)	1,782 (1.91)	35 (4.70)		469 (2.04)	464 (2.03)	5 (4.31)	
1500 – 2499 grams	25,290 (26.87)	25,169 (26.96)	121 (16.26)		6,821 (29.67)	6,803 (29.74)	18 (15.52)	
≥ 2500 grams	49,453 (52.55)	49,433 (52.95)	20 (2.69)	3208.05[b]	11,433 (49.73)	11,429 (49.96)	4 (3.45)	522.91

[a] 36 preterm births in the “No NEC” grouping in the model development set and 9 preterm births in the “No NEC” grouping in the validation set had no sex designation.

[b] $p < .001$

Table 2 (online only)

Comparison of acylcarnitines measured in preterm births with and without necrotizing enterocolitis: Model Development Cohort.

Acylcarnitine (nmol/mL)	No NEC Mean (SD)	NEC Mean (SD)	z =	*P* Value
log C2	3.27 (0.29)	3.25 (0.33)	-1.57	0.12
log C3	0.69 (0.43)	0.77 (0.53)	5.36	<.001
log C3DC	-2.56 (0.33)	-2.71 (0.33)	-12.10	<.001
log C4	-1.29 (0.53)	-0.96 (0.52)	17.98	<.001
log C5	-1.80 (0.60)	-1.09 (0.58)	29.35	<.001
log C5:1	-3.30 (0.60)	-3.12 (0.57)	7.63	<.001
log C5DC	-2.30 (0.38)	-2.38 (0.41)	-5.63	<.001
log C6	-2.67 (0.59)	-2.70 (0.60)	-1.58	0.11
log C8	-2.63 (0.60)	-2.47 (0.64)	6.66	<.001
log C8:1	-2.19 (0.59)	-2.10 (0.73)	3.27	0.001
log C10	-2.55 (0.59)	-2.82 (0.65)	-11.38	<.001
log C10:1	-2.82 (0.59)	-2.68 (0.74)	5.49	<.001
log C12	-1.87 (0.55)	-2.35 (0.60)	-21.85	<.001
log C14	-1.56 (0.46)	-1.86 (0.49)	-16.87	<.001
log C14:1	-2.11 (0.55)	-2.37 (0.53)	-12.51	<.001
log C16	0.78 (0.44)	0.33 (0.44)	-20.28	<.001
log C16:1	-1.74 (0.54)	-2.11 (0.58)	-17.41	<.001
log C18	-0.29 (0.36)	-0.35 (0.36)	-4.27	<.001
log C18:1	0.06 (0.34)	-0.08 (0.36)	-10.89	<.001
log C18:1OH	-3.54 (0.62)	-3.59 (0.60)	-1.80	0.07
log FC	3.77 (0.40)	4.00 (0.46)	14.08	<.001
log FC/(C16+C18:1)[a]	2.58 (0.45)	3.13 (0.46)	29.82	<.001
log C3/C2[a]	-2.58 (0.38)	-2.48 (0.43)	7.34	<.001

Abbreviations: SD, standard deviation.

[a]Ratio of acylcarnitine measurements in nmol/mL.

Table 3 (online only)

Association between per log unit increase in acylcarnitines/ratios and necrotizing enterocolitis.

	Adjusted OR[a]	95% CI	*P* Value
log C2	1.07	0.85 – 1.34	0.57
log C3	1.21	1.03 – 1.41	0.02
log C3DC	0.72	0.58 – 0.91	0.01
log C4	1.24	1.07 – 1.43	0.003
log C5	1.78	1.53 – 2.02	<.001
log C5:1	1.22	1.06 – 1.40	0.01
log C5DC	0.81	0.72 – 1.06	0.16
log C6	0.98	0.86 – 1.12	0.77
log C8	1.05	0.93 – 1.18	0.45
log C8:1	0.88	0.79 – 0.99	0.45
log C10	0.82	0.73 – 0.93	0.002
log C10:1	0.95	0.85 – 1.06	0.38
log C12	0.69	0.60 – 0.78	<.001
log C14	0.87	0.74 – 1.10	0.06
log C14:1	0.75	0.65 – 0.85	<.001
log C16	0.61	0.52 – 0.73	<.001
log C16:1	0.77	0.68 – 0.88	<.001
log C18	0.84	0.69 – 1.03	0.09
log C18:1	0.69	0.56 – 0.84	<.001
log C18:1OH	0.94	0.82 – 1.09	0.40
log FC	1.26	1.07 – 1.48	0.01
log FC/(C16+C18:1)	1.76	1.51 – 2.06	<.001
log C3/C2	1.25	1.04 – 1.57	0.02

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Adjusted for race/ethnicity, total parenteral nutrition, day at test (by grouping), and gestational age by birth weight grouping.

Table 4

Final acylcarnitine[a] (AC) necrotizing enterocolitis (AC-NEC) model.

AC log	C-5	C-5:1	C-8:1	C-12	C-14:1	FC/(C16+C18:1)	GA by BW grouping[b]	TPN
Odds Ratio[a]	1.75	1.25	0.72	0.74	0.66	1.38	1.84	1.35
95% CI	1.33 – 2.30	1.01 – 1.55	0.61 – 0.86	0.59 – 0.93	0.52 – 0.83	1.04 – 1.85	1.68 – 2.01	1.03 – 1.77
***P* Value**	<.001	0.04	<.001	0.01	<.001	0.03	<.001	0.03

Abbreviations: AC, acylcarnitine; BW, birth weight; CI, confidence interval; GA, gestational age; TPN, total parenteral nutrition.

[a]Odds ratios are per log unit increase in acylcarnitines, change in group for GA by BW, and TPN yes and no adjusted for all other factors in the model.

[b]< 32 weeks by < 1500 grams, 1500 to 2499 grams, and ≥ 2500 grams and 32 to 36 weeks by < 1500 grams, 1500 to 2499 grams, and ≥ 2500 gr

Table 5

Receiver operating characteristic curves (ROCs) for AC-NEC model[a].

	2005-2008 Model Development		2009 Validation	
	AUC	**95% CI**	**AUC**	**95% CI**
All	0.8983	0.8895 – 0.9072	0.9078	0.8903 – 0.9253
Weeks Gestation				
< 32	0.7406	0.7248 – 0.7564	0.7410	0.7021 – 0.7798
32 – 36	0.8600	0.8380 – 0.8819	0.9030	0.8725 – 0.9334
Hours/ Days at Testing				
12 hours – 2 days	0.9339	0.9238 – 0.9440	0.9518	0.9380 – 0.9655
3 – 4 days	0.8573	0.8361 – 0.8785	0.8898	0.8558 – 0.9239
5 – 6 days	0.7421	0.7057 – 0.7789	0.7329	0.6613 – 0.8045
7 – 8 days	0.7821	0.6928 – 0.8676	0.7512	0.5111 – 0.9913

Abbreviations: AC-NEC model, acylcarnitine necrotizing enterocolitis model; AUC, Area under the curve.

[a]log C:5, log C5:1, log C:8:1, log C12, log C14:1, log FC/(C16+C18:1), Gestational age by birth weight grouping, TPN.

Table 6

Receiver operating characteristic curves (ROCs) for AC-NEC [a] model by characteristics and acylcarnitines only and combined overall and by gestational age groupings.[a]

	Development		Validation	
	AUC	95% CI	AUC	95% CI
All				
GA by Birth Weight +TPN Only	0.8850	0.8751 – 0.8949	0.8914	0.8693 – 0.9135
Acylcarnitines (ACs) Only	0.8545	0.8429 – 0.8661	0.8784	0.8569 – 0.8999
GA by Birth Weight +TPN+ACs	0.8983	0.8895 – 0.9072	0.8983	0.8895 – 0.9072
<32 Weeks				
GA by Birth Weight +TPN Only	0.7239	0.7095 – 0.7382	0.7258	0.6975 – 0.7542
Acylcarnitines (ACs) Only	0.7188	0.7013 – 0.7363	0.7108	0.6688 – 0.7528
GA by Birth Weight +TPN+ACs	0.7406	0.7248 – 0.7564	0.7410	0.7021 – 0.7798
32 to 36 Weeks				
GA by Birth Weight +TPN Only	0.8263	0.8014 – 0.8511	0.8547	0.8037 – 0.9058
Acylcarnitines (ACs) Only	0.8121	0.7860 – 0.8382	0.8735	0.8302 – 0.9168
GA by Birth Weight +TPN+ACs	0.8600	0.8380 – 0.8819	0.9030	0.8725 – 0.9334

Abbreviations: AC, acylcarnitine; AC-NEC model, acylcarnitine necrotizing enterocolitis model; AUC, Area under the curve; CI, confidence interval; GA, gestational age; TPN, total parenteral nutrition.

[a] log C:5, log C5:1, log C:8:1, log C12, log C14:1, log FC/(C16+C18:1), Gestational age by birth weight grouping, TPN.