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Necrotizing enterocolitis is not associated with sequence variants in Anti-Oxidant Response genes in premature infants

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Abstract

Reactive oxygen species mediate intestinal injury in necrotizing enterocolitis (NEC), and yet the contribution of anti-oxidant response (ARE) gene polymorphisms to NEC risk remains unknown. Premature infants recruited in a multicenter study were genotyped for six ARE variants. Among 637 infants, 52 had NEC, and 22 developed surgical NEC. Gestational age < 28wk ($p<0.02$) and African American race ($p=0.03$) were associated with NEC. The *NFE2L2* (rs6721961), *SOD2* (rs4880), *GSTP1* (rs1695), *NQO1* (rs1800566), *GCLC* (rs17883901) and *HMOX1* (rs2071747) variants were not associated with medical or surgical NEC. This study does not support a role for common deleterious ARE variants in NEC.

Keywords

Anti-oxidant genes; polymorphisms; NEC; premature infants

INTRODUCTION

Necrotizing enterocolitis (NEC) is a severe form of intestinal disease in premature infants with a mortality of 20–35% (1,2). NEC is a multi-factorial disease with bowel ischemia,

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Venkatesh Sampath: Overall concept and design, supervision of experiments, data analysis, and drafting manuscript.
Neil Mulrooney, Jeffrey Murray, and John Dagle: Recruitment of study subjects, reviewed and revised the manuscript
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aberrant gut colonization, inflammation, and genetic factors known to contribute to disease pathogenesis (1,3,4). Studies in animal models and premature infants support a role for reactive oxygen species (ROS) in mediating inflammation and intestinal injury seen in NEC (1,5–8). Further, the immature antioxidant defenses of the preterm infant enhances vulnerability to diseases involving free radical injury such as NEC and chronic lung disease (6,9). An important protective mechanism against oxidative stress-induced tissue injury is the ubiquitous NF-E2-related factor-2 [Nrf2]-dependent antioxidant response elements [ARE] signaling pathway (10). Nrf2, encoded by the gene *NFEL2* is a transcription factor that is constitutively inhibited in the cytoplasm by being bound to Kelch like-ECH-associated protein 1 (10). Cellular oxidative stress liberates Nrf2 from the cytoplasm permitting nuclear translocation, where it transcriptionally induces antioxidant enzymes like superoxide dismutase 2 [SOD2], heme oxygenase 1 [HO1], NAD(P)H: quinone oxidoreductase 1 [NQO1], glutamate-cysteine ligase catalytic subunit [GCLC], and detoxification enzymes like glutathione S-transferases [GST] (10,11). We hypothesized that loss of function single nucleotide polymorphisms (SNPs) in the Nrf2-ARE axis genes will increase NEC risk in premature infants; this hypothesis was investigated in a prospective cohort study.

METHODS

Infant recruitment

Infants were recruited from neonatal nurseries at Children's Hospital of Wisconsin (Milwaukee, WI), Wheaton Franciscan Healthcare-St. Joseph's Hospital (Milwaukee, WI), Kosair's Children's Hospital (Louisville, KY), Children's Hospitals and Clinics of Minnesota (Minneapolis, MN), and University of Iowa Children's Hospital (Iowa City, IA) after institutional review board approval. After informed consent, 0.5mL of blood was collected, and shipped to Children's Hospital of Wisconsin where DNA extraction and genotyping was done. Clinical data was de-identified, assigned a study code and entered into a password-protected database.

Eligibility criteria

Premature infants born with a gestational age (GA) ≤ 35 weeks without major congenital anomalies of the heart, gastro-intestinal tract, kidney or lung were eligible.

Case Definition

NEC was defined as per modified Bell's criteria (12). Infants who had surgery for NEC were defined as having surgical NEC. Spontaneous ileal perforation was distinguished from NEC based on age of diagnosis, presence of pneumatosis, feeding history, and surgical information (when available). These infants were excluded from analysis.

Selection of Single nucleotide polymorphisms (SNPs)

Candidate ARE genetic variants were chosen based on; i) whether genes were transcriptionally activated by *NFE2L2*, ii) variants had a reported deleterious effect, and iii) mean allele frequency (MAF) > 2%.

Genotyping methods

Genomic DNA was extracted from blood samples using the FlexiGene DNA kit (Qiagen, Inc., Valencia, CA). The *NFE2L2* (rs6721961), *SOD2* (rs4880), *GSTP1* (rs1695), *NQO1* (rs1800566), *GCLC* (rs17883901) and *HMOX1* (rs2071747) SNPs were genotyped by performing a 5′ nuclease Taqman assay (Applied Biosystems, Foster City, CA) using predesigned TaqMan® SNP Genotyping Assay probes (ABI, Foster City, CA) essentially as done previously (13). Samples were analyzed on ABI HT7900 with SDS 2.3 software package. ***Quality control:*** 5% of the samples were re-genotyped by an independent technician blinded to prior results. There was >99% concordance for all samples.

Statistical analysis

Chi-square tests were used for comparisons between clinical variables among infants with and without NEC. We used genetic dominance and recessive models to examine NEC outcomes as studies suggests that ARE SNPs can exert dominant and recessive effects (14,15). Variant allele frequencies were compared among groups using the Pearson's Chi-square test or Fisher's exact test. Logistic regression was done to examine relationships between SNPs and clinical variables on NEC outcomes. ***Power***: A genetic dominance model was used for power calculations. Assuming a NEC incidence of 8% in our cohort, we estimated that a sample size of ~630 infants would give us 80% power with a p=0.05 to detect a 12 - 20 % difference in the prevalence of the variant allele between infants with and without NEC. SPSS 18.0 (SPSS Inc., Chicago, IL) and SAS 9.2 (SAS Inc., NC) were used for data analysis.

RESULTS

Epidemiology of NEC in our cohort

We prospectively recruited 644 Caucasian and African American infants born with gestational age (GA) ≤ 35 wk for this study. Seven infants were excluded from analysis; four died due to non-NEC related causes and genotyping was not completed in three infants. Among the 637 infants genotyped, NEC developed in 52 infants, 22 of whom had surgery for treatment of NEC. Birth-weight, GA, prenatal steroid use, proportion of males, type of feeding, rates of clinical chorioamnionitis and clinically significant patent ductus arteriousus were not significantly different among infants with or without NEC (Table 1). The proportion of infants with GA <28 wk (p=0.02) and of African American origin (p=0.03) were higher among infants with NEC, while there was a trend towards a greater proportion of infants with birth weight <1000g among the NEC cohort (Table 1).

Relationship between ARE-pathway variants and NEC

Hardy-Weinberg equilibrium was confirmed at all loci. The genotype frequencies of ARE genetic variants among infants without NEC, all NEC and surgical NEC is shown in table 2. We used both genetic dominant and genetic recessive models for analysis. The *SOD2 (rs4880), GSTP1 (rs1695), NFE2L2 (rs*6721961*), NQO1* (rs1800566), *GCLC* (rs17883901) and *HMOX1* (rs2071747) SNPs were not associated with increased NEC in our cohort (Table 2). In logistic regression models for NEC that included clinical variables and genetic

variants only GA<28 wk (Odds ratio 1.9, 95% Confidence interval 1.1-3.4, p=0.03) was associated with NEC. Infants of African American race showed a trend towards increased NEC (p=0.07).

Among the 52 infants with NEC, 22 developed surgical NEC (data on surgical NEC was unavailable in 5 infants). Among the clinical/epidemiological variables examined, only GA was associated with NEC. Compared to infants without surgical NEC, infants with surgical NEC had a lower gestational age (26.8 ± 3.0 wk vs. 27.9 ± 2.5 wk, p=0.05). The *SOD2, GSTP1, NFE2L2, NQO1, GCLC* and *HMOX1* genetic variants were not associated with increased surgical NEC.

DISCUSSION

NEC is a complex disease influenced by interactions between genetic factors, intestinal microbiota, host immune responses and intestinal ischemia (1,3). Reactive oxygen species are implicated in the pathogenesis of NEC, and yet, whether variants in genes that mediate cytoprotective responses against free radical injury contribute to NEC has not been investigated (1,6). In this study we pursued a pathway approach to investigate the impact of functional SNPs in Nrf2-ARE axis genes on NEC susceptibility. We did not find an association between any of the six variants investigated and medical or surgical NEC. Although we examined only selective SNPs in ARE genes, these data do not support a role for common (MAF>2%) Nrf2-ARE genetic variants in NEC. Use of a genetically heterogeneous population and a small sample size for infants with surgical NEC are limitations of this study. Additional considerations which might have impacted our results include heterogeneity in NEC diagnosis, inadequate power to examine NEC by race, and issues arising from population stratification (4).

The role of ROS in NEC is supported by animal and human studies (5–7,16). Perrone et al. (5) showed that cord blood levels of total hydroperoxides and advanced oxidation products were elevated in premature infants who developed NEC. In rodent models of NEC, the use of retinoic acid or N-acetylcysteine attenuates intestinal injury (7,16). *NFE2L2* encodes Nrf2, which transcriptionally activates anti-oxidant enzymes that protect against free radical injury (10). The promoter SNP examined in this study (rs6721961;-617C>A) decreases Nrf2 expression, and increases the risk of acute lung injury following sepsis in adults (17). Arisawa et al. (18) reported that promoter *NFE2L2* variants were associated with increased inflammatory bowel disease (IBD) in adults. We did not find an association between a hypomorphic promoter variant and NEC in this study. While both IBD and NEC are linked to aberrant host-microbiota interactions, NEC is an acute disease occurring in the setting of an immature immune system and bowel ischemia. We speculate that partial Nrf2 deficiency arising from a heterozygous state of this variant does not modulate the anti-oxidant activity sufficiently to contribute to NEC. The *GCLC* variant (-129C/T) examined decreases expression of the catalytic sub-unit of glutamate cysteine ligase, a key enzyme regulating intracellular glutathione synthesis (19,20). The lack of association between this variant and NEC could potentially be explained by limited penetrance in a heterozygous state and the unclear relevance of intestinal glutathione in preventing NEC.

The *NQO1* (rs1800566, p.P187S) variant investigated in this study abolishes NQO1's ability to catalyze two electron reduction of cellular substrates in the homozygous state (21). The *SOD2* (rs4880, p.A16V) variant results in decreased manganese superoxide dismutase activity (22). While variants in these genes have not been investigated in the context of NEC, the *NQO1* variant, but not the SOD2 variant was reported to be associated with severity of ulcerative colitis in adults (15). Neither of these variants were associated with NEC or surgical NEC in our cohort. *GSTP1* encodes glutathione S-transferase Pi 1, an enzyme that detoxifies electrophile compounds using glutathione, while *HMOX1* encodes inducible heme oxygenase that plays an important role in cellular anti-oxidant response (23,24). The missense *GSTP1* (rs1695, p.I1047V) and *HMOX1* (rs2071747, p.D7H) variants that decrease function of respective proteins were not associated with NEC in our cohort (24,25). Potential explanations for these negative results could be redundancy and overlap in the function of these proteins in the gut, preservation of some protein function with presence of variants and variation in developmental expression of these proteins in the intestine. Studies by Ozdemir et al.(16) and Tayman et al.(8) have shown that chemically mediated inhibition of ROS attenuates NEC in rodent models. Further, mice deficient in HMOX1 have increased intestinal injury with experimental NEC (26). While these studies support a pathogenic role for ROS in NEC they do not demonstrate ROS initiate injury in NEC. The pathophysiology of NEC centers on aberrant activation of innate immune signaling by microbiota in the intestine. It is likely that excessive ROS production is triggered by noxious stimuli, which then contribute to inflammation and cell death. Therefore, genetic variants that modulate ROS homeostasis such as ARE variants might have limited effects on the phenotype.

In summary, this is the first study to examine the impact of functional polymorphisms in anti-oxidant genes on NEC susceptibility in a preterm infant cohort. These results suggest that common SNPs in the Nrf2-ARE axis do not alter NEC vulnerability in premature infants. While early studies indicated that innate immune and cytokine gene variants (see Supplement for tabulation of prior studies) may modulate NEC susceptibility follow up studies have either not confirmed the initial results or have not been done (2,27–29). This remains a limitation of genetic association studies investigating NEC. Our previous study showed an association between an NFKB1 variant and NEC, but we have not pursued follow up studies because the high prevalence of this variant in infants without NEC (65%) clearly suggests that it is not a causal variant (29). Therefore, our recent efforts have focused on ARE variants and genes that inhibit immune signaling such as SIGIRR (30). Use of sequencing-based approaches to query rare and common variants in adequately powered studies is a topic for future research in NEC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Bibliography

1. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med. 2011; 364:255–64. [PubMed: 21247316]
2. Gordon P, Christensen R, Weitkamp J-H, Maheshwari A. Mapping the New World of Necrotizing Enterocolitis (NEC): Review and Opinion. EJ Neonatol Res. 2012; 2:145–72. [PubMed: 23730536]
3. Afrazi A, Sodhi CP, Richardson W, et al. New insights into the pathogenesis and treatment of necrotizing enterocolitis: Toll-like receptors and beyond. Pediatric research. 2011; 69:183–8. [PubMed: 21135755]
4. Harding D. Impact of common genetic variation on neonatal disease and outcome. Arch Dis Child Fetal Neonatal Ed. 2007; 92:F408–13. [PubMed: 17712190]
5. Perrone S, Tataranno ML, Negro S, et al. May oxidative stress biomarkers in cord blood predict the occurrence of necrotizing enterocolitis in preterm infants? J Matern Fetal Neonatal Med. 2012; 25(Suppl 1):128–31. [PubMed: 22339378]
6. Perrone S, Tataranno ML, Santacroce A, Negro S, Buonocore G. The role of oxidative stress on necrotizing enterocolitis in very low birth weight infants. Curr Pediatr Rev. 2014; 10:202–7. [PubMed: 25088341]
7. Ozdemir R, Yurttutan S, Sari FN, et al. Antioxidant effects of N-acetylcysteine in a neonatal rat model of necrotizing enterocolitis. J Pediatr Surg. 2012; 47:1652–7. [PubMed: 22974601]
8. Tayman C, Tonbul A, Kosus A, et al. N-acetylcysteine may prevent severe intestinal damage in necrotizing enterocolitis. J Pediatr Surg. 2012; 47:540–50. [PubMed: 22424351]
9. Asikainen TM, White CW. Pulmonary antioxidant defenses in the preterm newborn with respiratory distress and bronchopulmonary dysplasia in evolution: implications for antioxidant therapy. Antioxidants & redox signaling. 2004; 6:155–67. [PubMed: 14713347]
10. Cho HY, Reddy SP, Kleeberger SR. Nrf2 defends the lung from oxidative stress. Antioxidants & redox signaling. 2006; 8:76–87. [PubMed: 16487040]
11. Cho HY, Kleeberger SR. Genetic mechanisms of susceptibility to oxidative lung injury in mice. Free radical biology & medicine. 2007; 42:433–45. [PubMed: 17275675]
12. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatric clinics of North America. 1986; 33:179–201. [PubMed: 3081865]
13. Sampath V, Garland JS, Helbling D, et al. Antioxidant response genes sequence variants and BPD susceptibility in VLBW infants. Pediatr Res. 2015; 77:477–83. [PubMed: 25518008]
14. Forsberg L, de Faire U, Morgenstern R. Oxidative stress, human genetic variation, and disease. Arch Biochem Biophys. 2001; 389:84–93. [PubMed: 11370676]
15. Kosaka T, Yoshino J, Inui K, et al. Involvement of NAD(P)H:quinone oxidoreductase 1 and superoxide dismutase polymorphisms in ulcerative colitis. DNA Cell Biol. 2009; 28:625–31. [PubMed: 19715479]
16. Ozdemir R, Yurttutan S, Sari FN, et al. All-trans-retinoic acid attenuates intestinal injury in a neonatal rat model of necrotizing enterocolitis. Neonatology. 2013; 104:22–7. [PubMed: 23615357]
17. Marzec JM, Christie JD, Reddy SP, et al. Functional polymorphisms in the transcription factor NRF2 in humans increase the risk of acute lung injury. Faseb J. 2007; 21:2237–46. [PubMed: 17384144]
18. Arisawa T, Tahara T, Shibata T, et al. Nrf2 gene promoter polymorphism is associated with ulcerative colitis in a Japanese population. Hepatogastroenterology. 2008; 55:394–7. [PubMed: 18613373]
19. Koide S, Kugiyama K, Sugiyama S, et al. Association of polymorphism in glutamate-cysteine ligase catalytic subunit gene with coronary vasomotor dysfunction and myocardial infarction. J Am Coll Cardiol. 2003; 41:539–45. [PubMed: 12598062]
20. Siedlinski M, Postma DS, van Diemen CC, Blokstra A, Smit HA, Boezen HM. Lung function loss, smoking, vitamin C intake, and polymorphisms of the glutamate-cysteine ligase genes. Am J Respir Crit Care Med. 2008; 178:13–9. [PubMed: 18420959]

21. Siegel D, Anwar A, Winski SL, Kepa JK, Zolman KL, Ross D. Rapid polyubiquitination and proteasomal degradation of a mutant form of NAD(P)H:quinone oxidoreductase 1. Mol Pharmacol. 2001; 59:263–8. [PubMed: 11160862]
22. Sutton A, Imbert A, Igoudjil A, et al. The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. Pharmacogenet Genomics. 2005; 15:311–9. [PubMed: 15864132]
23. Choi AM, Alam J. Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. Am J Respir Cell Mol Biol. 1996; 15:9–19. [PubMed: 8679227]
24. Zimniak P, Nanduri B, Pikuła S, et al. Naturally occurring human glutathione S-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. Eur J Biochem. 1994; 224:893–9. [PubMed: 7925413]
25. Tanaka G, Aminuddin F, Akhabir L, et al. Effect of heme oxygenase-1 polymorphisms on lung function and gene expression. BMC Med Genet. 2011; 12:117. [PubMed: 21902835]
26. Schulz S, Wong RJ, Jang KY, et al. Heme oxygenase-1 deficiency promotes the development of necrotizing enterocolitis-like intestinal injury in a newborn mouse model. Am J Physiol Gastrointest Liver Physiol. 2013; 304:G991–1001. [PubMed: 23578787]
27. Szebeni B, Szekeres R, Rusai K, et al. Genetic polymorphisms of CD14, toll-like receptor 4, and caspase-recruitment domain 15 are not associated with necrotizing enterocolitis in very low birth weight infants. Journal of pediatric gastroenterology and nutrition. 2006; 42:27–31. [PubMed: 16385250]
28. Heninger E, Treszl A, Kocsis I, Derfalvi B, Tulassay T, Vasarhelyi B. Genetic variants of the interleukin-18 promoter region (-607) influence the course of necrotising enterocolitis in very low birth weight neonates. European journal of pediatrics. 2002; 161:410–1. [PubMed: 12174825]
29. Sampath V, Le M, Lane L, et al. The NFKB1 (g-24519delATTG) variant is associated with necrotizing enterocolitis (NEC) in premature infants. J Surg Res. 2011; 169:e51–7. [PubMed: 21529841]
30. Sampath V, Menden H, Helbling D, et al. SIGIRR Genetic Variants in Premature Infants With Necrotizing Enterocolitis. Pediatrics. 2015; 135:e1530–4. [PubMed: 25963006]

What is known about this subject?

- NEC isc a severe intestinal inflammatory disease affecting premature infants
- Reactive oxygen species contribute to inflammation and tissue injury in NEC
- Anti-oxidant response genes orchestrate cytoprotective responses to free radical injury

What are the new findings and/or what is the impact on clinical practice?

- Initial study to examine impact of anti-oxidant gene polymorphisms in NEC susceptibility
- Common deleterious variants in anti-oxidant genes were not associated with NEC risk
- Study provides impetus to investigate immune or other genetic pathways as susceptibility loci for NEC

Table 1
Distribution of epidemiological risk-factors for NEC in our cohort

Data is represented as mean ± standard deviation or as raw numbers (n) with percentages (%).

Variable	Infants without NEC (n=585)	Infants with NEC (n=52)	P Value
Gestational age (wk)	28.0 ± 2.4	27.4 ± 3.4	0.30
Birth-weight (grams)	1053 ± 287	1153 ± 703	0.31
Race - Caucasians	459 (78%)	34 (65%)	0.03
African American	126 (22%)	18 (35%)	
Prenatal steroid use	511 (88%)	45 (87%)	0.80
Male sex	305 (52%)	32 (62%)	0.19
Clinical chorioamnionitis	48 (9%)	5 (10%)	0.85
Patent ductus arteriosus	220 (38%)	23 (44%)	0.35
Infants <28 weeks	236 (40%)	30 (58%)	0.02
Infants <1000 grams	242 (41%)	28 (54%)	0.08
Type of feeding *			
Breast milk	135 (42.2%)	24 (53.3%)	0.33
Formula milk	78 (24.4%)	10 (22.2%)	
Breast and formula milk	107 (33.4%)	11 (24.5%)	

* Feeding information was not available from one center.

Table 2
Distribution of ARE genetic variants by NEC outcomes in our cohort

Genotype frequencies of study subjects stratified by BPD outcome are presented. rs number; reference SNP accession ID number.

Variant rs number	No NEC (n=585) genotype frequency (%)	NEC (n=52) genotype frequency (%)	Surgical NEC (n=22) genotype frequency (%)
GSTP1 rs1695	A/A - 242 (41.2) A/G - 256 (44) G/G - 86 (14.8)	A/A - 20 (38.5) A/G - 24 (46.1) G/G - 8 (15.4)	A/A - 10 (45.5) A/G - 8 (36.3) G/G - 4 (18.2)
SOD2 rs4880	T/C - 140 (24) C/T - 297 (50.8) C/C - 146 (25.2)	T/C - 14 (27.5) C/T - 29 (56.8) C/C - 8 (15.7)	T/C - 5 (22.7) C/T - 12 (54.6) C/C - 5 (22.7)
NQO1 rs1800566	C/C - 358 (61.2) C/T - 203 (34.8) T/T - 23 (4.0)	C/C - 32 (61.5) C/T - 19 (36.6) T/T - 1 (1.9)	C/C - 15 (68.2) C/T - 7 (31.8) T/T - 0 (0)
NFE2L2 rs6721961	C/C - 457 (78.3) C/A - 122 (21) A/A - 4 (0.7)	C/C - 40 (77) C/A - 11 (21.1) A/A - 1 (1.9)	C/C - 17 (77.3) C/A - 5 (21.2) A/A - 0 (1.5)
GCLC rs17883901	C/C - 492 (84.2) C/T - 88 (15.1) T/T - 4 (0.7)	C/C - 44 (84.6) C/T - 7 (13.5) T/T - 1 (1.9)	C/C - 19 (86.4) C/T - 2 (9.1) T/T 1 - 1 (4.5)
HMOX1 rs2071747	G/G - 533 (91.6) G/C - 46 (7.9) C/C - 3 (0.5)	G/G - 46 (88.5) G/C - 6 (11.5) C/C - 0 (0)	G/G - 18 (81.8) G/C - 4 (18.2) C/C - 0 (0)