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Single nucleotide polymorphisms in the dual specificity phosphatase genes and risk of necrotizing enterocolitis in premature infant

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Abstract

BACKGROUND: Differences in the susceptibility of preterm infants to develop necrotizing enterocolitis (NEC) implicate potential genetic differences in response to the inflammatory stimuli leading to NEC. Dual specificity phosphatases (DUSPs) are a key suppressor pathway of the mitogen-activated protein kinase (MAPK) pro-inflammatory signaling pathway. We hypothesized that inherited single nucleotide polymorphisms (SNPs) in DUSP genes contribute to NEC susceptibility in premature infants.

METHODS: Patients admitted between 2010 and 2015 born at < 32 weeks GA and≤ 1,500 g BW with stage II+NEC (cases; $n = 50$) and age, weight-matched controls ($n = 38$) were included. Blood samples were collected for DNA isolation. Agena Mass Array assay was used to examine 31 SNPs in 9 different DUSP genes. Calculated minor allele frequencies (MAF) for cases and controls were compared using χ^2 and logistic regression.

RESULTS: The presence of the rs704074 SNP was associated with a 48% decreased risk of developing NEC (OR 0.52; 95% CI 0.27–1.01, $p = 0.04$). The odds of surgical NEC decreased by 78% (OR 0.22; 95% CI 0.06–0.84, $p = 0.027$) for each copy of rs704074/G allele in patients with NEC.

CONCLUSION: In this small single-center pilot study, DUSP-6 SNP (rs704074) was associated with a lower risk of developing NEC and surgical NEC, the most severe form of NEC, in preterm infants.

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Disclosure

The authors have nothing to disclose.

Keywords

Biomarker; neonate; MKP; MAPK

1. Introduction

Necrotizing enterocolitis (NEC) is the leading cause of death from gastrointestinal disease in premature neonates, affecting up to 20% of preterm babies, with mortality as high as 50% [1]. The overall incidence of NEC in premature infants has remained constant between 5–10% for the last several decades [2]. Although several risk factors have been shown to contribute to NEC development such as gut prematurity, abnormal microbiome [3], tissue ischemia from congenital heart disease or vascular insufficiency [4], and formula feedings [5] the exact pathogenesis still remains unclear. With the exception of prematurity, epidemiologic studies have not been able to identify clear, consistent risk factors that increase the susceptibility to NEC in this population [6]. This gap in knowledge has significantly hindered the development of preventative and curative therapies resulting in a significant burden of disease in premature infants.

NEC is a complex gastrointestinal disease characterized by an exaggerated inflammatory response leading to early cell death and intestinal barrier dysfunction [7]. Genetic susceptibility to develop this exaggerated inflammatory response in premature infants may account for the overall small percentage of preterm infants that develop disease despite exposure to similar environments and risk factors. Dysregulation of immune signaling, specifically Toll-like receptor (TLR)-4 signaling, have been implicated in the pathogenesis of NEC [8-10]. An important innate immune pathway is the mitogen-activated protein kinase (MAPK) pathway, a key pro-inflammatory signaling cascade activated via TLR4 activation by microbial stimulation. Our interest lies in negative immune regulators (i.e. those that limit the inflammatory response) [11] that could lead to novel methods of modulating the exaggerated inflammatory response seen in NEC. Dual specificity phosphatases (DUSPs), also referred to as mitogen-activated protein kinase phosphatases (MKPs), are such a family of protein phosphatases that negatively regulate the immune response by dephosphorylating (inactivating) MAPKs. The MAPK of particular interest are p38, extracellular-signal regulating kinase (ERK), and c-Jun-N-terminal kinase (JNK) all known to induce the production of inflammatory mediators [12, 13] and known targets of various DUSP family members. Our group has shown that DUSP1 (MKP-1) deficiency increased proinflammatory cytokine production in intestinal epithelial cells as well as increased inflammation-induced apoptosis setting the stage for barrier dysfunction [14]. Based on this work, we hypothesized that the presence of single nucleotide polymorphisms (SNPs) in the DUSP genes are differentially enriched in preterm infants that develop NEC versus those that do not develop NEC. To test this hypothesis, we investigated 31 DUSP SNPs in premature infants that either developed NEC or did not develop NEC.

2. Methods

2.1. Infant enrollment

The Institutional Review Board at Nationwide Children's Hospital approved this study. Neonates were enrolled from the neonatal intensive care unit at Nationwide Children's Hospital from January 1, 2010 to December 31, 2015. Subjects were enrolled in the Perinatal Research Repository (PRR) and the Ohio Perinatal Research Network (OPRN). Inclusion criteria included premature infants born at < 32 weeks gestational age (GA) and < 1500 g birth weight (BW) with Stage 2 or greater NEC and enrolled in the PRR. Exclusion criteria included infants with congenital gastrointestinal malformations, genetic, or metabolic disorders. Clinical data collected from chart review included: demographics, presence or absence of antenatal steroids, chorioamnionitis, breastmilk feeding or formula, and NEC staging. Feeding type was classified and recorded as the feeds being received at the time of NEC diagnosis.

2.2. NEC definitions

Using the Vermont Oxford Network definition, NEC was diagnosed either by direct observation of intestine at surgery or pathological examination postmortem or by using a set of strict clinical criteria. A clinical diagnosis of NEC was made based on at least one physical finding (bilious gastric aspirate or emesis, abdominal distention, or occult/gross blood in the stool in the absence of anal fissure) and at least one radiographic finding (pneumatosis intestinalis, hepatobiliary gas, or pneumoperitoneum) [15]. Medical NEC is defined as stage II or greater of the Bell's staging criteria [16], which is managed medically with antibiotics, NPO, and supportive care. Surgical NEC is defined as Stage III or greater of the Bell's staging criteria, which requires surgical intervention, as is the case for pneumoperitoneum or free air in the peritoneum from an intestinal perforation. Surgical NEC patients included all those infants that required surgical intervention to resolve NEC-related symptoms.

2.3. Study cohort

The initial study cohort included a total of 118 patients who were < 32 weeks gestational age and/or<1,500 g birthweight. However, since the referenced minor allele frequencies (MAFs) of the selected DUSP SNPs are highly enriched in Caucasian ancestry, those with African and Asian ancestry ($n = 30$) were not included in the present study. The final study cohort included 88 patients, with 38 controls and 50 cases (Stage II+NEC). Further classification of the NEC cases included 30 patients with medical NEC and 20 with surgical NEC.

2.4. Genotyping

After consent was obtained, a blood sample was collected and maintained on ice for no more than six hours before centrifugation. The blood sample was centrifuged and components stored for DNA extraction. A total of 200 ng of DNA per sample was extracted and assayed for 31 SNPs in 9 DUSP genes by the High Throughput Genotyping and Sequencing Core at the Abigail Wexner Research Institute at Nationwide Children's Hospital. SNP genotyping was performed using the Agena MassARRAY system, which is based on matrix-assisted

laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Agena Bioscience, San Diego, CA). In this highly multiplexed assay, alternate alleles for a given loci produce single-allele base extension reaction products of different masses that are resolved by MALDI-TOF MS. All primers and probes were designed into 7 plates using the Agena Assay Design Suite v2.0.

2.5. SNP selection

Initial DUSP SNP query was conducted using the National Center for Biotechnology Information (NCBI) SNP database for known DUSP genes implicated in human disease. The final groups of selected SNPs were verified with the SNAP software: SNP Annotation and Proxy Search. SNAP finds proxy SNPs based on linkage disequilibrium, physical distance, and/or membership in commercial genotyping arrays (http://archive.broadinstitute.org/mpg/snap/). The selected SNPs were < 1Kbp from the 5'end and > 1Kbp from the 3'end. The minor allele frequency (MAF) for the selected SNPs were > 0.2 but < 0.5 global MAF as reported by the NCBI.

2.6. Statistics

Demographic and clinical characteristics were compared between NEC cases and non-NEC cases (controls) groups. Continuous variables were compared using Student's *t*-test. Categorical variables were compared using the χ^2 test. SNPs were analyzed for Hardy-Weinberg Equilibrium using Stata/IC 13.0 (College Station, Texas). Calculated MAF of cases and controls were compared using the Fisher's exact test. The distributions of genotypes for the DUSP6 SNP rs704074 were compared between control, medical NEC, and surgical NEC patients using the Fisher's exact test. Simple logistic regression was used to estimate the odds of developing any NEC or surgical NEC. In an additive model of logistic regression, surgical NEC was compared to medical NEC, for each additional minor allele (G) in the DUSP6 SNP rs704074. A p-value of < 0.05 was considered statistically significant. This study was designed to test a specific hypothesis and only candidate genes in the DUSP family were included in the study. Given the limitation of study sample size, less conservative values are also of interest, and may be hypothesis generating. Therefore, no correction was made for multiple testing. Prism GraphPad statistical software (San Diego, CA), SAS version 9.4 (Cary, NC), and Stata/IC 13.0 (College Station, Texas) were used to complete all analyses in this study.

3. Results

3.1. Study population and characteristics

Table 1 compares demographic and clinical characteristics between case and control groups. Overall, there were no significant differences in antenatal or postnatal characteristics between case and control groups. Among infants that developed NEC, the majority of infants developed medical NEC (60%) as compared to surgical NEC (40%).

3.2. Association between DUSP SNPs and NEC

Table 2 compares the calculated MAF for non-NEC controls and the calculated MAF for NEC cases for the 31 SNPs in 9 DUSP genes studied. We found that the rs704074 SNP (G

minor allele) was less common in cases (MAF = 0.19) than in controls (MAF = 0.33). The presence of the rs704074 SNP was associated with a 48% lower risk of developing NEC (OR 0.52; 95% CI 0.27–1.01, $p = 0.04$). Two other SNPs (rs10744 and rs704073) in the DUSP6 gene were found to have a lower MAF in cases than in the controls, however this was not found to be statistically significant. Table 3 contains genotypic distribution data for all 31 DUSP SNPs. All SNPs were analyzed for Hardy-Weinberg Equilibrium and found to be in equilibrium.

3.3. Allele frequencies for rs704074 SNP

Figure 1 graphs the allele frequencies for the rs704074 DUSP6 SNP which found a lower minor allele (G) frequency in the surgical NEC group when compared to control ($p = 0.003$), and also when compared to the medical NEC group ($p = 0.02$). There was no difference in the minor allele (G) frequency between the control and medical NEC group. Further analysis of the DUSP6 SNPs rs704074, rs704073, and rs10744 in patients that developed medical or surgical NEC, demonstrated a significantly lower risk of developing surgical NEC for each of these three DUSP6 SNPs ($p < 0.05$) (Table 4). In a logistic regression with the rs704074 using an additive logistic regression model with surgical and medical NEC as dependent variables, we found that for each minor allele (G) in the genotype the odds for a medical NEC patient to develop surgical NEC decreased by 78% (OR 0.22 [95% CI 0.06–0.84]; $p = 0.027$).

4. Discussion

The major objective of this study was to investigate the association of SNPs in DUSP genes and the risk of developing NEC in premature infants. The major findings of this study were that 1) in our cohort the rs704074 SNP in the DUSP6 gene was associated with a 48% decreased risk of developing any type of NEC, 2) all three DUSP6 SNPs had statistically significantly lower MAF in patients that developed surgical NEC compared to those that developed medical NEC, and 3) in patients who developed medical NEC, the odds of developing surgical NEC (the most severe form of NEC) decreased by 78% for every copy of the minor allele (G) of the rs704074 SNP that was present. These novel preliminary findings support the hypothesis that SNPs in DUSP genes can alter susceptibility to developing NEC in premature infants.

While the exact mechanism of the pathogenesis of NEC remains unknown, the NICHD defined NEC as “an uncontrolled exuberant inflammatory response to bacterial colonization that characterizes the intestine of premature infants” [17]. Based on this definition, we chose to focus on the family of DUSPs for their known regulatory nature of the innate immune response. In the present study, we found the calculated MAF for rs704074 SNP in the DUSP6 gene in premature infants that developed NEC to be lower than in premature infants that did not develop NEC. DUSP6 also known as mitogen activated protein kinase phosphatase (MKP)-3 is a cytoplasmic phosphatase that preferentially negatively regulates ERK1/2 by decreasing the intensity and duration of ERK activity. DUSP6 accomplishes this by serving as a chaperone and anchor for ERK1/2 between the cytoplasm and nucleus preventing activation of pro-inflammatory genes [11, 13, 18, 19]. The inflammatory

properties of ERK1/2 signaling include stimulation of TNF-α production in macrophages, T-cell differentiation, and modulation of plasma cell differentiation [19]. ERK1/2 also plays a critical role in cell survival, cell-cycle regulation, differentiation and proliferation [20, 21]. DUSP6 is a known strong negative regulator of ERK signaling, influencing the innate inflammatory response, cellular survival, and proliferation, which are all critical components that contribute to the intestinal epithelial barrier dysfunction that characterizes NEC.

Human intestinal expression of DUSP6 protein has been shown to predominantly reside in the epithelium, specifically the cytoplasm and membrane of epithelial/glandular cells [22]. Evidence of the role of DUSP6 in intestinal inflammation has been shown in mouse models of colitis. Investigators demonstrated worsening colonic inflammation in IL-$10^{-/-}$/$DUSP6^{-/-}$ double-knockout mice compared to IL-$10^{-/-}$ mice in the form of high degrees of epithelial crypt hyperplasia, globlet cell depletion, and infiltration of mononuclear cells in the colonic lamina propria [23]. These same investigators demonstrated that IL-$10^{-/-}$/$DUSP6^{-/-}$ colonic tissue expressed significantly greater levels of pro-inflammatory cytokines (IFN-γ, TNF-α) compared to IL-$10^{-/-}$ colonic tissue [23]. Recently, Ruan et. al. demonstrated in a diet-induced model of obesity that the intestinal microbiome of DUSP6-deficient mice regulated the homeostasis between the barrier function, mucosal immunity, and microbiota [24]. These studies highlight the important role DUSP6 plays in regulating intestinal inflammation, mucosal immunity, and shaping the microbiome, all critical components to the development of NEC. Regulation of intestinal inflammation in NEC involves many genes, including nucleotide-binding oligomerization domains (NOD) 2 [25, 26] and SIGIRR [27] that effect toll-like receptors (TLR) signaling and modify NEC risk.

SNPs are naturally occurring variation in the genome involving a single nucleotide base change. SNPs are located in different regions such as promoters, exons, introns, and 5’or 3’ untranslated regions (UTRs) [28]. The rs704074/G, rs704073/T, and rs10744/T SNPs are all located in the 3’UTR of the DUSP6 gene found in chromosome 12q22-23 [29]. The 3’UTRs regulate gene expression through mRNA degradation and translation. As a regulatory region, the 3’UTR is critical for normal gene expression such that SNPs in the 3’UTR can alter mRNA degradation and protein translation [30]. Investigators have found strong associations between a variety of other DUSP-6 SNPs and non-small cell lung cancer patient survival and treatment efficacy [31, 32]. However, functional impact of rs704074/G, rs704073/T, and rs10744/T SNPs on the DUSP6 protein are unknown. Thus, we postulate that the rs704074 SNP, which in our cohort is enriched in patients that did not develop NEC, may be a gain of function mutation that results in greater DUSP6 activity by way of 3’UTR translational regulation, limiting ERK-dependent pro-inflammatory signaling. Further studies are needed to elucidate the exact mechanism by which rs704074 confers protection against the development of NEC.

There are several limitations of this study that should be noted. This is a single-center pilot study with a relatively small sample size, and we did not adjust for multiple comparisons of the SNPs. A candidate gene approach was used to detect a significant difference in the rs704074 SNP in the DUSP6 gene between premature infants that developed NEC and those that did not develop NEC during their NICU hospitalization. Epidemiological studies have shown higher NEC rates in African-American infants, making population genetics and

genetic ancestry important considerations in NEC studies [33, 34]. We acknowledge that this study needs to be replicated in a larger independent cohort to validate these findings. Another limitation is the inherent complexity of the disease itself and the difficulty to define the genetics of this multifaceted inflammatory bowel disease.

Our findings suggest that in our preterm cohort, genetic variations in the DUSP6 gene are associated with the development of NEC. Our study provides important data that supports a more detailed investigation into the functional impact of the DUSP-6 SNPs in intestinal inflammation, cellular apoptosis, and proliferation. This study highlights the need for genetic markers that either predict the development or NEC or confer protection to NEC, and specifically surgical NEC, in order to decrease morbidity and mortality in this fragile preterm population. Future studies will determine if DUSP-6 therapy, through its known roles of regulating intestinal inflammation, mucosal immunity, and shaping the microbiome, might ameliorate and/or prevent this potentially devastating gastrointestinal disease.

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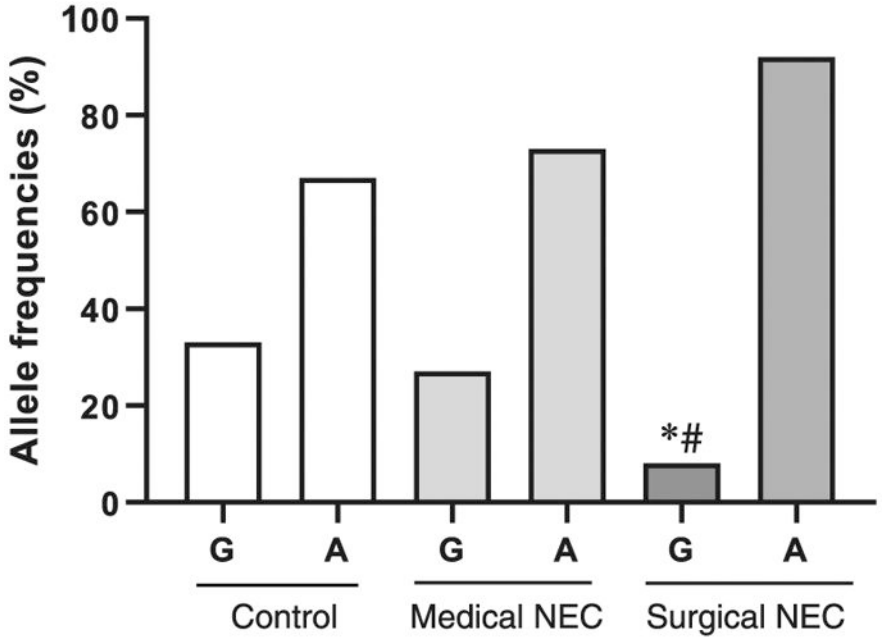

Fig. 1.

Allele frequencies for the rs704074 SNP in the DUSP6 gene. G is the minor allele (SNP), and A is the major allele (wild-type). The allele frequency is calculated as a percentage of minor or major alleles present in each patient group. *Surgical NEC ($n = 20$) different from control ($n = 38$) ($p = 0.003$), #Surgical NEC different from medical NEC ($n = 30$) ($p = 0.02$). *P*-values determined by Fisher's exact test.

Table 1

Demographic and clinical characteristics

Characteristics	Non-NEC controls (*n* = 38)	NEC-cases (*n* = 50)	*p*-value
Gestational age (weeks)	26 ± 1	26 ± 2	0.83
Birth weight (grams)	807 ± 215	812 ± 241	0.92
Gender-male	17 (45)	29 (58)	0.22
Ethnicity:			
Hispanic	0	1 (2)	0.39
Non-Hispanic	*36 (95)	49 (98)	
Antenatal steroids	*28 (78)	*43 (90)	0.14
Chorioamnionitis	4 (11)	6 (12)	0.83
Feedings:			
Breastmilk	#14 (42)	&16 (34)	0.40
Formula	19 (58)	31 (66)	0.45
NEC type:			
Medical		30 (60)	
Surgical		20 (40)	

Abbreviation: NEC = necrotizing enterocolitis. Continuous variables presented as mean ± SD and analyzed using Student's *t*-test. Categorical characteristics presented as number (percentage), analyzed using chi-square test.

*missing data *n* = 2

#missing data *n* = 5

&missing data *n* = 3.

Table 2

DUSP SNPs and calculated minor allele frequencies

Gene	SNP	Minor allele	Calculated MAF controls (*n* = 38)	Calculated MAF NEC cases (*n* = 50)	**p*-value
DUSP1	rs881150	A	0.21	0.29	0.30
DUSP1	rs881152	A	0.21	0.30	0.23
DUSP1	rs322351	T	0.42	0.38	0.64
DUSP1	rs322380	A	0.34	0.39	0.53
DUSP1	rs322382	T	0.41	0.37	0.64
DUSP1	rs3805476	A	0.12	0.15	0.66
DUPS2	rs2969489	A	0.43	0.47	0.65
DUSP2	rs1724121	G	0.33	0.33	1
DUSP4	rs567436	T	0.29	0.32	0.74
DUSP4	rs569209	A	0.25	0.32	0.32
DUSP4	rs474824	C	0.38	0.51	0.10
DUSP5	rs1042606	A	0.30	0.40	0.21
DUSP5	rs1889568	G	0.32	0.35	0.75
DUSP5	rs2282239	G	0.32	0.35	0.75
DUSP5	rs3793892	G	0.33	0.40	0.35
DUSP5	rs915216	C	0.29	0.39	0.20
DUSP6	rs10744	T	0.30	0.18	0.07
DUSP6	rs704073	T	0.30	0.18	0.07
DUSP6	rs704076	T	0.25	0.17	0.26
DUSP6	rs769700	C	0.26	0.22	0.59
DUSP6	rs704074	G	0.33	0.19	0.05
DUSP7	rs4687607	A	0.26	0.23	0.72
DUSP7	rs3821841	T	0.04	0.05	1
DUSP7	rs9851576	G	0.22	0.20	0.71
DUSP9	rs7883986	T	0.21	0.23	0.86
DUSP9	rs4243539	T	0.50	0.46	0.65
DUSP9	rs7882888	A	0.17	0.16	0.84
DUSP10	rs10746413	C	0.42	0.44	0.88
DUSP10	rs10779454	T	0.32	0.33	0.87
DUSP16	rs4255620	G	0.47	0.49	0.88
DUPS16	rs4763833	C	0.41	0.42	0.88

Abbreviations: DUSP = Dual Specificity Phosphatase; SNP = single nucleotide polymorphism; MAF = minor allele frequency.

*Two-sided *p*-value obtained by Fisher's exact test. Thirty-one SNPs evaluated in nine DUSP genes (DUSP 1, 2, 4, 5, 6, 7, 9,10, 16).

Table 3

DUSP SNP distribution

SNP	Control N(%)	NEC-Medical N(%)	NEC-Surgical N(%)
rs881150	2(5), 12(32), 24(63)	2(7), 15(50), 13(43)	3(15), 4(20), 13(65)
rs881152	2(5), 12(32), 24(63)	2(7), 10(33), 18(60)	3(15), 10(50), 7(35)
rs322351	7(18), 18(47), 13(34)	4(13), 15(50), 11(37)	5(25), 5(25), 10(50)
rs322380	2(5), 22(58), 14(37)	3(10), 12(40), 15(50)	3(15), 15(75), 2(10)
rs322382	8(22), 15(39), 15(39)	4(13), 15(50), 11(37)	4(20), 6(30), 10(50)
rs3805476	0(0), 9(24), 29(76)	0(0), 9(30), 21(70)	0(0), 6(30), 14(70)
rs2969489	8(21), 17(45), 13(34)	6(20), 18(60), 6(20)	5(25), 7(35), 8(40)
rs1724121	4(10), 17(45), 17(45)	4(13), 15(50), 11(37)	0(0), 10(50), 10(50)
rs567436	4(10), 14(37), 20(53)	1(3), 18(60), 11(37)	2(10), 8(40), 10(50)
rs569209	2(5), 15(39), 21(55)	1(3), 18(60), 11(37)	2(10), 8(40), 10(50)
rs474824	7(18), 15(40), 16(42)	6(20), 19(63), 5(17)	5(25), 10(50), 5(25)
rs1042606	2(5), 19(50), 17(45)	4(13), 17(57), 9(30)	4(20), 7(35), 9(45)
rs1889568	4(10), 16(42), 18(47)	3(10), 16(53), 11(37)	3(15),7(35), 10(50)
rs2282239	4(11), 16(42), 18(47)	3(10), 16(53), 11(37)	3(15), 7(35), 10(50)
rs3793892	2(5), 21(55), 15(40)	3(10), 12(40), 15(50)	4(20), 14(70), 2(10)
rs915216	2(6), 18(47), 18(47)	3(10), 18(60), 9(30)	4(20), 7(35), 9(45)
rs10744	4(11), 15(39), 19(50)	2(7), 12(40), 16(53)	0(0), 2(10), 18(90)
rs704073	4(11), 15(39), 19(50)	2(7), 12(40), 16(53)	0(0), 2(10), 18(90)
rs704076	4(11), 11(29), 23(60)	2(7), 12(40), 16(53)	0(0), 1(5), 19(95)
rs769700	3(8), 14(37), 21(55)	0(0), 10(33), 20(67)	2(10), 8(40), 10(50)
rs704074	6(16), 13(34), 19(50)	1(3), 14(47), 15(50)	1(5), 1(5), 18(90)
rs4687607	2(5), 16(42), 20(53)	2(7), 8(27), 20(66)	2(10), 7(35), 11(55)
rs3821841	0(0), 3(8), 35(92)	0(0), 5(17), 25(83)	0(0), 0(0), 20(100)
rs9851576	2(5), 13(34), 23(61)	5(17), 9(30), 16(53)	0(0), 1(5), 19(95)
rs7883986	4(11), 8(21), 26(68)	7(23), 7(23), 16(54)	0(0), 2(10), 18(90)
rs4243539	16(42), 6(16), 16(42)	10(33), 9(30), 11(37)	8(40), 1(5), 11(55)
rs7882888	4(11), 5(13), 29(76)	2(7), 6(20), 22(73)	3(15), 0(0), 17(85)
rs10746413	5(13), 22(58), 11(29)	6(20), 9(30), 15(50)	5(25), 13(65), 2(10)

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SNP	Control N(%)	NEC-Medical N(%)	NEC-Surgical N(%)
rs10779454	5(13), 14(37), 19(50)	6(20), 12(40), 12(40)	0(0), 9(45), 11(55)
rs4255620	9(24), 18(47), 11(29)	6(20), 15(50), 9(30)	8(40), 6(30), 6(30)
rs4763833	6(16), 19(50), 13(34)	5(17), 16(53), 9(30)	5(25), 6(30), 9(45)

*Data presented as minor allele homozygote, heterozygote, major allele homozygote.

Table 4

Association of DUSP6 SNPs and risk of surgical NEC

SNP-rs	Minor Allele Allele	Calculated medical NEC MAF (*n* = 30)	Calculated surgical NEC MAF (*n* = 20)	OR (95% CI)	*p*-value
rs704074	G	0.27	0.08	0.21 (0.05, 0.84)	0.012 *
rs704073	T	0.27	0.07	0.14 (0.03, 0.68)	0.003 *
rs10744	T	0.27	0.07	0.14 (0.03, 0.68)	0.003 *

Abbreviations: DUSP = Dual Specificity Phosphatase; NEC = necrotizing enterocolitis; SNP = single nucleotide polymorphism; MAF = minor allele frequency.

* p-value obtained by logistic regression.