SIGIRR gene variants in term newborns with congenital heart defects and necrotizing enterocolitis

Ekaterina Konstantinovna Zaikova[1], Aleksandra Vladimirovna Kaplina[2], Natalia Aleksandrovna Petrova[2], Tatiana Mikhailovna Pervunina[3], Anna Aleksandrovna Kostareva[4], Olga Viktorovna Kalinina[2,5]

[1]World-Class Research Centre for Personalized Medicine, Almazov National Medical Research Centre, Research Laboratory of Autoimmune and Autoinflammatory Diseases, St. Petersburg, Russia, [2]Almazov National Medical Research Centre, Research Laboratory of Physiology and Diseases of Newborns, St. Petersburg, Russia, [3]Almazov National Medical Research Centre, Institute of Perinatology and Pediatrics, St. Petersburg, Russia, [4]Almazov National Medical Research Centre, Institute of Molecular Biology and Genetics, St. Petersburg, Russia, [5]Department of Laboratory Medicine and Genetics, Institution of Medical Education, Almazov National Medical Research Centre, St. Petersburg, Russia

ABSTRACT

Background : **Necrotizing enterocolitis (NEC) is a common gastrointestinal emergency among neonates which is characterized by acute intestinal inflammation and necrosis. The main risk factors for NEC are prematurity, low birth weight, and some preexisting health conditions such as congenital heart defects (CHDs). Investigation of the potential genetic predisposition to NEC is a promising approach that might provide new insights into its pathogenesis. One of the most important proteins that play a significant role in the pathogenesis of NEC is Toll-like receptor 4 (TLR4) which recognizes lipopolysaccharide found in Gram-negative bacteria. In intestinal epithelial cells, a protein encoded by the *SIGIRR* gene is a major inhibitor of TLR4 signaling. A few *SIGIRR* variants, including rare p.Y168X and p.S80Y, have already been identified in preterm infants with NEC, but their pathogenic significance remains unclear. This study aimed to investigate the spectrum of *SIGIRR* genetic variants in term newborns with CHD and to assess their potential association with NEC.**

Methods and Results : **A total of 93 term newborns with critical CHD were enrolled in this study, 33 of them developed NEC. *SIGIRR* genetic variants were determined by Sanger sequencing of all exons. In total, eight *SIGIRR* genetic variants were identified, two of which were found only in newborns with NEC ($P = 0.12$). The rare missense p.S80Y (rs117739035) variant in exon 4 was found in two infants with NEC stage IIA. Two infants with NEC stage III and stage IB carried a novel duplication c. 102_121dup (rs552367848) variant in exon 10 that has not been previously associated with any clinical phenotype.**

Conclusions : **The presence of both variants only in neonates who developed NEC, together with earlier published data, may suggest their potential contribution to the risk of developing NEC in term infants with CHD and allow planning larger cohort studies to clarify their relevance.**

Keywords : c. 102_121dup variant, congenital heart disease, necrotizing enterocolitis, p.S80Y variant, *SIGIRR*

Access this article online

Quick Response Code:

Website: https://journals.lww.com/aopc

DOI: 10.4103/apc.apc_30_23

How to cite this article: Zaikova EK, Kaplina AV, Petrova NA, Pervunina TM, Kostareva AA, Kalinina OV. *SIGIRR* gene variants in term newborns with congenital heart defects and necrotizing enterocolitis. Ann Pediatr Card 2023;16:337-44.

Address for correspondence: Dr. Olga Viktorovna Kalinina, Department of Laboratory Medicine and Genetics, Almazov National Medical Research Centre, 2 Akkuratova St., St. Petersburg 197341, Russia.
E-mail: olgakalinina@mail.ru

Submitted: 01-Mar-2023 **Revised:** 28-Mar-2023 **Accepted:** 03-Aug-2023 **Published:** 01-Apr-2024

INTRODUCTION

Necrotizing enterocolitis (NEC) is the most common pathology of the gastrointestinal tract in the neonatal period, characterized by acute intestinal inflammation and necrosis. Despite intensive study of NEC pathogenesis and various treatment approaches to NEC, the morbidity, survival, and mortality rates have not globally changed over the past decades.[1] The incidence rate of NEC in preterm and very low birth weight newborns is up to 13%[2] and the mortality rate is up to 50%.[3] Full-term neonates, however, rarely develop NEC, with an incidence of 10%–12% among all newborns with NEC.[4,5]

NEC is a multifactorial disease with an unclear pathogenesis. The main risk factors for the development of NEC are prematurity, low birth weight, formula feeding, abnormal bacterial colonization of the gut, and intestinal ischemia/hypoxia.[1,6,7] The likelihood of developing NEC is inversely proportional to gestational age, with preterm infants at far greater risk than full-term neonates.[8,9] Among full-term infants, one of the major predisposing factors for NEC is congenital heart disease (CHD), in particular, critical CHDs, which require early surgical intervention.[10-13] Besides, CHD is characterized by bowel hypoperfusion and ischemia and is associated with systemic inflammation in the perioperative period that may trigger the development of NEC.[14-18]

One of the key components of innate immunity that contribute to the development of NEC is Toll-like receptor 4 (TLR4), a pathogen recognition molecule, that recognizes lipopolysaccharide (LPS) in Gram-negative bacteria and induces a pro-inflammatory response.[19-21] It is believed that TLR4 signaling is activated not only in response to microbial invasion but also during nonmicrobial processes such as ischemia.[22] The latter was hypothesized to play a crucial role in the pathogenesis of NEC in term newborns with CHD.[15] It was shown *in vivo* that induction of experimental NEC in mice by the combination of formula feeding, hypoxia, and LPS administration significantly elevated the level of TLR4 expression in the intestinal epithelium of mice.[23-25] Moreover, Sodhi *et al.* demonstrated that TLR4-deficient mice were protected from NEC.[23]

Several studies highlight the role of genetics in the pathogenesis of NEC.[26-29] One of the potential gene candidates that may contribute to NEC susceptibility is a single-immunoglobulin interleukin (IL)-1-related receptor (*SIGIRR*, NC_000011.10, Gene ID: 59307), a negative regulator of the intestinal TLR4 signaling. Mice deficient in SIGIRR demonstrate unregulated activation of the intestinal TLR4, followed by elevated intestinal inflammation and enhanced enterocyte apoptosis.[30] Sampath *et al.* identified a few *SIGIRR* variants, including stop mutation p.Y168X, rare missense variants p.S80Y, p.P115R, and splice region variant (rs201897529), in preterm infants with NEC, that may affect the TLR4-mediated inflammation.[31]

Most up-to-date research focused on genetic predisposition to NEC in preterm infants. In this study, we assessed the contribution of the *SIGIRR* variants to a genetic background underlying NEC in term infants with critical CHD and revealed eight genetic variants including a novel duplication c.102_121dup (rs552367848) variant in exon 10 at the 3′ untranslated region (UTR).

MATERIALS AND METHODS

Patients

A total of 93 term newborns with critical CHD and a birth weight of >2500 g and/or a gestational age >37 weeks were enrolled in this study. The diagnosis of NEC was based on both clinical signs and abnormal abdominal ultrasound and radiographs; the severity of NEC was classified according to the modified Bell staging criteria.[32] The spectrum of congenital heart defects included: hypoplastic left heart syndrome, double-inlet left ventricle, unbalanced (RV dominant) atrioventricular septal defect, tricuspid atresia, double-outlet right (single) ventricle, transposition of great arteries, coarctation of the aorta, pulmonary atresia/stenosis, aortic valve stenosis, tetralogy of Fallot, double-outlet right ventricle, interrupted aortic arch, and Ebstein's anomaly.

The exclusion criterion was refusal to participate in the study for any personal reason. This research was approved by the local institutional ethics committee (protocol no. 1702-21, February 15, 2021) and complied with the Helsinki Declaration. Informed consent was obtained from parents or legal representatives of all participating neonates.

DNA extraction

Genomic DNA was extracted from peripheral blood samples using FlexiGene DNA Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The quality and quantity of extracted DNA were assessed using NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA was stored at –20°C until use.

SIGIRR gene amplification and sequencing

Primers were designed to amplify and sequence all 10 exons of *SIGIRR* gene using the Primer-BLAST designing tool[33] and are listed in Supplemental Table 1. The polymerase chain reaction (PCR) reaction mix (40 μL) contained 100 ng of extracted genomic DNA, Green GoTaq Reaction Buffer (Promega, Madison, USA), 0.2 mM each dNTP, 0.4 μM each primer, and Taq polymerase (Thermo

Table 1: Distribution of congenital heart defects in the study cohort

Type of CHD	Total	No NEC, *n* (%)	NEC, *n* (%)	Type of operation		
				No NEC (*n*)	NEC (*n*)	NEC in postoperative period (*n*)
Anomalous LCA from the PA	2	0	2 (6)	0	Reimplantation of LCA in the aortic root, PA reconstruction (2)	2
Single-ventricle CHD						
Hypoplastic left heart syndrome	10	9 (15)	1 (3)	Norwood procedure (7/6*) DKS procedure (1) Hybrid procedure (1)	Norwood procedure (1*)	1
Double-inlet left ventricle	6	2 (3)	4 (12)			3
+ Pulmonary atresia/stenosis	4	1 (2)	3 (9)	mBTS placement (1)	mBTS placement (3)	2
+ CoA	2	1 (2)	1 (3)	DKS procedure (1*)	Norwood procedure (1)	1
Unbalanced (RV dominant) atrioventricular septal defect	5	2 (3)	3 (9)			2
+ Obstructed supracardiac TAPVR	1	0	1 (3)	0	Pulmonary vein stenting (1)	1
+ Pulmonary atresia/stenosis	2	0	2 (6)	0	mBTS placement (1) No surgery (1)	1
+ CoA	2	2 (3)	0	Modified Amato technique (1) Norwood procedure (1*)	0	0
Tricuspid atresia	3	2 (3)	1 (3)	mBTS placement (1) No surgery (1)	Mitral valve repair, atrioseptectomy (1)	0
Double-outlet right (single) ventricle	2	1 (2)	1 (3)	No surgery (1)	PA banding (1)	1
Total	26	16 (27)	10 (30)			
Two-ventricle CHD						
Transposition of the great arteries	22	16 (27)	6 (18)	ASO (13/2*) ASO + modified Amato technique (1) ASO + ascending aorta repair (1*) mBTS placement (in LVOT obstruction) (1)	mBTS placement (in LVOT obstruction) (1**) ASO (5)	1 3
CoA	20	15 (25)	5 (15)	Modified Amato technique (6) AAR in CoA with aortic arch hypoplasia (5) End-to-end anastomosis (4)	Modified Amato technique (3) End-to-end anastomosis (2)	3 1
Pulmonary atresia/stenosis	11	5 (8)	6 (18)	mBTS placement (3) mBTS placement + infundibulectomy (1) Antegrade palliation (1)	RV to PA conduit (1) mBTS placement (4) Antegrade palliation (1)	1 4 1
Aortic valve stenosis	4	2 (3)	2 (6)	Aortic commissurotomy (2)	Aortic commissurotomy (2)	1
TOF	3	2 (3)	1 (3)	Complete repair of TOF (2)	Complete repair of TOF (1)	1
Double-outlet RV	2	2 (3)	0	Infundibulectomy+widening of RVOT with patch + PA plasty with patch (1) Infundibulectomy + widening of RVOT with patch (1)	0	0
Interrupted aortic arch and ventricular septal defect	2	1 (2)	1 (3)	AAR + PA banding (1)	AAR + extended septal myectomy of LVOT obstruction + commissuroplasty of tricuspid valve + PA banding (1*)	1
Ebstein`s anomaly	1	1 (2)	0	No surgery (1)	0	
Total	65	44 (73)	21 (64)			

*Mortality related to acute heart failure in CHD, **Mortality related to NEC. Modified Amato technique - Surgical reconstruction in CoA with hypoplastic distal aortic arch, complete repair of TOF – Patch closure of ventricular septal defect, infundibulectomy, widening of the RVOT with placement of patch. TOF: Tetralogy of Fallot, CHD: Congenital heart defect, NEC: Necrotizing enterocolitis, LCA: Left coronary artery, PA: Pulmonary artery, DKS: Damus–Kaye–Stansel procedure, mBTS: Modified Blalock–Taussig shunt, TAPVR: Total anomalous pulmonary venous return, ASO: Arterial switch operation, CoA: Coarctation of the aorta, RV: Right ventricle, RVOT: Right ventricular outflow tract, LVOT: Left ventricular outflow tract, AAR: Aortic arch reconstruction

Fisher Scientific, Waltham, USA). Due to high GC content in exons 6–8, 1 M betaine (Sigma-Aldrich, St. Louis, USA) was added to the PCR reaction mix to improve the amplification of these exons. The thermal profile of the PCR included an initial denaturation for 5 min at 95°C, followed by 40 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 59°C, and elongation for 1 min at 72°C followed by the final extension at 72°C for 5 min. All amplification steps were performed in a Veriti Thermal Cycler (Applied Biosystems, Waltham, USA).

The sequencing reactions were performed using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, USA) and analyzed on a 3500 Genetic Analyzer (Applied Biosystems, Waltham, USA). The obtained sequences were analyzed using SnapGene software version 3.2.1 (GSL Biotech LLC, Chicago, IL, USA) with the alignment to NC_000011.10: C417455-405716 as a reference sequence.

The clinical significance of all identified *SIGIRR* genetic variants was evaluated according to guidelines of the American College of Medical Genetics and Genomics[34] and information available from public databases.

Statistical analysis

Categorical variables are expressed as numbers and percentages, and continuous variables as medians and interquartile ranges. For categorical variables, the two-tailed Fisher's exact test and Kruskal–Wallis analysis of variance test were used to assess differences between NEC newborns and newborns without NEC. For continuous variables, differences between groups were detected using the Mann–Whitney *U*-test. All statistical analyses were performed using Statistica 10.0 (StatSoft, USA), and $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

Out of all enrolled newborns, 65 (70%) infants had a two-ventricle CHD, 26 (28%) a single-ventricle CHD, and 2 (2%) an anomalous left coronary artery from the pulmonary artery [Table 1]. The most prevalent diagnoses were transposition of the great arteries (22/93; 24%), coarctation of the aorta (20/93; 22%), pulmonary atresia/stenosis (11/93; 12%), and hypoplastic left heart syndrome (10/93; 11%). The median birth weight and gestational age were 3300 g (interquartile range: 3010–3660) and 39 weeks (interquartile range: 38–40), respectively.

In total, 33 infants were diagnosed with NEC: 26 of them developed NEC after cardiac surgery (1 infant had NEC stage III, 2 – NEC stage IIB, 22 – NEC stage IIA, and 1 – NEC stage IB) and 7 in the preoperative period (all had NEC stage IIA). The median patient age at NEC diagnosis was 14 days (interquartile range: 7–24).

Thirteen infants, out of all enrolled infants, died in the first 2 months of life, including 3 with NEC. Mortality was associated with acute heart failure, whereas one infant's death was predominantly associated with NEC stage III that required abdominal surgery. The majority of deaths were among neonates with hypoplastic left heart syndrome who underwent the Norwood procedure.

The most prevalent CHD diagnoses in newborns with NEC were both transposition of the great arteries (6/33; 18%) and pulmonary atresia/stenosis (6/33; 18%), followed by coarctation of the aorta (5/33, 15%) and double-inlet left ventricle (4/33, 12%) [Table 1]. Besides, both infants with an anomalous left coronary artery from the pulmonary artery and all two with unbalanced (RV dominant) atrioventricular septal defect combined with pulmonary atresia/stenosis developed NEC stage IIA. However, there were no differences in gender, birth weight, gestational age, route of delivery, place of residence, or Apgar score between newborns with or without NEC [Table 2].

SIGIRR genetic variants

Identification of *SIGIRR* genetic variants in all 10 exons was performed for 60 patients with CHD; of those, 21 were diagnosed with NEC. Rare variants in *SIGIRR* were detected in 4 patients, and all of them belonged to NEC group. A rare missense p.S80Y (rs117739035) variant in exon 4 [displayed in Figure 1a] was detected in 2 out of 60 neonates, and both developed NEC stage IIA. Infants harboring the p.S80Y variant were diagnosed with coarctation of the aorta and anomalous left coronary artery from the pulmonary artery and underwent the modified Amato technique[35] and reimplantation of the LCA in the aortic root with PA reconstruction, respectively. One infant with coarctation of the aorta who underwent the modified Amato technique and developed NEC stage IB, and one infant with transposition of the great arteries who underwent the mBTS placement (in LVOT obstruction) and developed NEC stage III with a fatal outcome, carried a duplication c.102_121dup (NM_021805.3; rs552367848) variant in exon 10 at the 3′ UTR [displayed in Figure 1b] – a variant not previously associated with clinical phenotype. None of the newborns had the rare pathogenic p.Y168X (rs766709278), the likely benign p.P115R (rs111819059), or the splice region variant (rs201897529) previously reported in preterm infants with NEC.[31]

The majority of newborns (59/60) carried benign missense p.Q312R variant (rs3210908) in exon 9, and simultaneously two synonymous substitutions c.945 T > G (p.P315P; rs3087588) and c.1086 C > T (p.V362V; rs7947) in exons 9 and 10, respectively. Moreover, 39

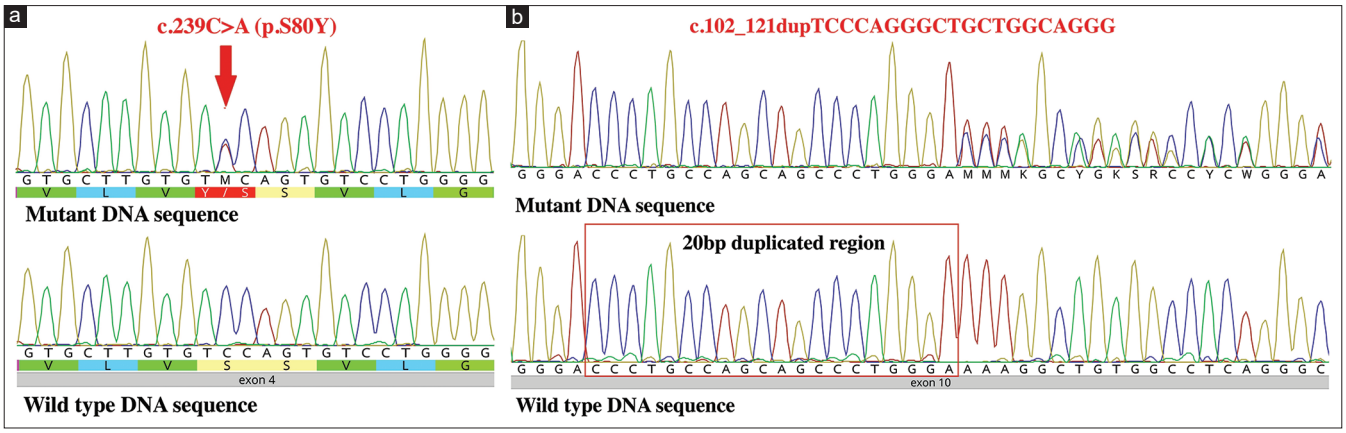

Figure 1: *SIGIRR* genetic variants identified only in newborns with CHD and necrotizing enterocolitis. (a) Single-nucleotide substitution in exon 4 resulting in p.S80Y variant. (b) 20bp duplication in exon 10 at the 3′ untranslated region

Table 2: Characteristics of newborns with critical congenital heart defect enrolled in the study

Newborns' characteristic (*n*)	Diagnosis		*P*
	No NEC (*n*=60), *n* (%)	NEC (*n*=33), *n* (%)	
Gender			
Female (41)	23 (38)	18 (55)	0.19
Male (52)	37 (62)	15 (45)	
Gestational age, week (93)	39 (38–40)	39 (38–40)	0.64
Birth weight, g (93)	3330 (2975–3740)	3300 (3090–3620)	0.83
Route of delivery			
Unassisted vaginal delivery (67)	41 (68)	26 (79)	0.31
Assisted vaginal delivery (5)	4 (7)	1 (4)	
Cesarean section (21)	15 (25)	6 (18)	
Place of residence			
North Caucasian federal district (40)	28 (47)	12 (36)	0.39
Northwestern federal district (53)	32 (53)	21 (64)	
Apgar score at 1 min (93)	7 (7–7)	7 (7–7)	0.39
Apgar score at 5 min (93)	8 (7–8)	8 (8–8)	0.06

NEC: Necrotizing enterocolitis

of them were homozygous for all three variants. Two infants (one with NEC) had synonymous c.768 C > T (p.P256P; rs112731199) substitution in exon 8, and one infant had two synonymous substitutions c.612 C > G (p.L204 L; rs199995236) in 6 exon and c.798 C > G (p.R266R; rs199670238) in 8 exon. None of the 60 patients carried nucleotide changes in exons 1, 2, 3, 5, and 7 of the *SIGIRR* gene compared to the reference genome.

Since the p.S80Y and c.102_121dup variants were identified only in newborns with NEC, 33 newborns with CHD, 11 of whom developed NEC, were additionally enrolled in this study to assess the contribution of these *SIGIRR* genetic variants to the development of NEC. However, none of the infants carried either the missense variant p.S80Y in exon 4 or the duplication c.102_121dup in exon 10. In addition, none of the 11 newborns with NEC had the rare pathogenic p.Y168X variant.

DISCUSSION

In this study, we evaluated an association between *SIGIRR* genetic polymorphic variants and the development of NEC in full-term newborns with critical CHD. In total, eight genetic variants of the *SIGIRR* gene were identified within five out of ten exons, two of which (p.S80Y and c.102_121dup) were found only in infants with NEC [Table 3]. However, distribution frequencies did not reach a statistical significance ($P = 0.12$), probably because of the insufficient number of newborns enrolled in the study.

The duplication c.102_121dup (rs552367848) in exon 10 is a novel variant that has not been previously reported in connection to any clinical phenotype. Considering that the duplication is located at the 3′ UTR region of the *SIGIRR* gene, this variant may potentially play a role in the posttranscriptional regulation of gene expression by changing the binding sites for microRNAs or some RNA-binding proteins, which in turn could impact TLR4-mediated inflammation.[36] According to Ensembl release 108,[37] the duplicated sequence is predicted to contain CTCF-binding site (s) for the transcription factor CTCF, which can act as a transcriptional repressor, activator, insulator-binding protein or enhancer-blocking protein. Further functional studies are needed to

Table 3: Identified *SIGIRR* genetic variants in newborns with congenital heart defect

Genetic variants	CHD	CHD + NEC	gnomAD allele frequency	Classification according to ACMG	Mutation taster	Grantham score	PolyPhen-2	*P*
c. 239C>A (p.S80Y) rs117739035	-	2	0.0285	Benign	Benign	144	Probably damaging: 0.999	0.12
c. 612 C>G (p.L204L) rs199995236	1	-	0.000884	Likely benign	Benign	NA	NA	1.00
c. 768C>T (p.P256P) rs112731199	1	1	0.0262	Benign	Benign	NA	NA	1.00
c. 798C>G (p.R266R) rs199670238	1	-	0.000819	Likely benign	Benign	NA	NA	1.00
c. 935A>G (p.Q312R) rs3210908	38	21	0.838	Benign	Benign	43	Benign: 0.000	1.00
c. 945T>G (p.P315P) rs3087588	38	21	0.821	Benign	Benign	NA	NA	1.00
c. 1086C>T (p.V362V) rs7947	38	21	0.833	Benign	Benign	NA	NA	1.00
c. 102_121dup* rs552367848	-	2	0.00896	Benign	Benign	NA	NA	0.12

*Mortality in one newborn predominantly associated with NEC. CHD: Congenital heart defect, NEC: Necrotizing enterocolitis, NA: Not applicable, ACMG: American College of Medical Genetics and Genomics

evaluate the impact of the novel duplication variant c. 102_121dup on the *SIGIRR* gene expression and protein synthesis.

The missense p.S80Y variant (rs117739035) in exon 4, found only in infants with NEC in our study, was previously identified in extremely low birth weight twins with NEC stages II and III who simultaneously carried a stop variant p.Y168X (rs766709278).[31] The following functional analyses, performed on a human embryonic kidney cell line by transfecting with a plasmid encoding both variants (p.Y168X and p.S80Y), demonstrated that the presence of a mutated *SIGIRR* leads to abolished protein function and subsequent NF-κB activation as well as IL-8, IL-6, and iNOS expression, which are all markers of inflammation. The p.S80Y variant was associated with kidney function[38] and the level of circulated apolipoprotein A-I, a major component of high-density lipoprotein, that is known to be connected with cardiovascular diseases[39,40] based on GWAS, as well as also suggested to be a potentially causal for diabetic nephropathy in the Finnish population.[41] However, more detailed functional studies of the missense variant p.S80Y are needed to uncover its role.

Several studies demonstrate that newborns with hypoplastic left heart syndrome,[10,42-45] truncus arteriosus,[10] and atrioventricular septal defects[46] are at a higher risk of developing NEC compared to other CHDs. In our study among infants with NEC, only 3% (1/33) had hypoplastic left heart syndrome and 9% (3/33) had unbalanced atrioventricular septal defect while the most prevalent diagnoses were transposition of the great arteries (6/33; 18%) and pulmonary atresia/stenosis (6/33; 18%), probably due to sample size. Moreover, the majority of newborns with both unbalanced atrioventricular septal defect and double-inlet left ventricle and with NEC in this study also had pulmonary atresia/stenosis. Interestingly, it was previously noted that the surgical procedure selected to repair CHD may contribute significantly to the risk of developing NEC, in particular, neonates who underwent the Norwood procedure for hypoplastic left heart syndrome are at far greater risk for NEC of all CHD patients.[45,47] However, in the present study, the highest incidence of NEC occurred among newborns following modified Blalock–Taussig shunt placement (27%), whereas only in 6% of cases NEC developed after the Norwood operation. Furthermore, several studies noted significant differences in Apgar score[15] and gestational age[48] between NEC and no-NEC infants with CHD, while in our cohort, this was not the case.

To our knowledge, this is the first study to identify genetic variants in all 10 exons of *SIGIRR* gene in term newborns with congenital heart defects that are at risk of developing NEC. The obtained results did not reveal a major impact of *SIGIRR* genetic variants in the development of NEC in the term infant with critical CHD. However, the presence of the p.S80Y and c.102_121dup variants only in neonates who developed NEC, including one with fatal NEC stage III, together with earlier published data, may suppose their potential contribution to the risk of developing NEC in infants with CHD. Expanding the cohort groups would be useful to clarify the clinical relevance of both genetic variants.

Our study has several limitations, such as insufficient sample size, lack of promoter region, or intronic regions sequencing of the *SIGIRR* gene, which could potentially affect the function of the gene. In addition, functional studies of the variants need to be performed to uncover the detailed role of the variants described.

CONCLUSIONS

Despite the absence of statistical significance ($P = 0.12$), the presence of both variants only in neonates who

developed NEC, together with earlier published data, may suggest their potential contribution to the risk of developing NEC in term infants with CHD and allow planning larger cohort studies to clarify their relevance.

Financial support and sponsorship

This work was supported by the Ministry of Health of Russian Federation № 121031100287-8.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Niño DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: New insights into pathogenesis and mechanisms. Nat Rev Gastroenterol Hepatol 2016;13:590-600.
2. Alsaied A, Islam N, Thalib L. Global incidence of necrotizing enterocolitis: A systematic review and meta-analysis. BMC Pediatr 2020;20:344.
3. Henry MC, Moss RL. Neonatal necrotizing enterocolitis. Semin Pediatr Surg 2008;17:98-109.
4. Abbo O, Harper L, Michel JL, Ramful D, Breden A, Sauvat F. Necrotizing enterocolitis in full term neonates: Is there always an underlying cause? J Neonatal Surg 2013;2:29.
5. Frid G, Reppucci M, Lum T, Paul M, Seiden H, Coakley BA. Comparison of necrotizing enterocolitis in pre-mature infants versus. Term-born infants with congenital heart disease. Front Pediatr 2021;9:802607.
6. Schnabl KL, Van Aerde JE, Thomson AB, Clandinin MT. Necrotizing enterocolitis: A multifactorial disease with no cure. World J Gastroenterol 2008;14:2142-61.
7. Caplan MS, Jilling T. New concepts in necrotizing enterocolitis. Curr Opin Pediatr 2001;13:111-5.
8. Gregory KE, Deforge CE, Natale KM, Phillips M, Van Marter LJ. Necrotizing enterocolitis in the premature infant: Neonatal nursing assessment, disease pathogenesis, and clinical presentation. Adv Neonatal Care 2011;11:155-64.
9. Gephart SM, McGrath JM, Effken JA, Halpern MD. Necrotizing enterocolitis risk: State of the science. Adv Neonatal Care 2012;12:77-87.
10. McElhinney DB, Hedrick HL, Bush DM, Pereira GR, Stafford PW, Gaynor JW, *et al.* Necrotizing enterocolitis in neonates with congenital heart disease: Risk factors and outcomes. Pediatrics 2000;106:1080-7.
11. Pickard SS, Feinstein JA, Popat RA, Huang L, Dutta S. Short- and long-term outcomes of necrotizing enterocolitis in infants with congenital heart disease. Pediatrics 2009;123:e901-6.
12. Kelleher ST, McMahon CJ, James A. Necrotizing enterocolitis in children with congenital heart disease: A literature review. Pediatr Cardiol 2021;42:1688-99.
13. Bolisetty S, Lui K, Oei J, Wojtulewicz J. A regional study of underlying congenital diseases in term neonates with necrotizing enterocolitis. Acta Paediatr 2000;89:1226-30.
14. Kashif H, Abuelgasim E, Hussain N, Luyt J, Harky A. Necrotizing enterocolitis and congenital heart disease. Ann Pediatr Cardiol 2021;14:507-15.
15. van der Heide M, Mebius MJ, Bos AF, Roofthooft MT, Berger RM, Hulscher JB, *et al.* Hypoxic/ischemic hits predispose to necrotizing enterocolitis in (near) term infants with congenital heart disease: A case control study. BMC Pediatr 2020;20:553.
16. Lazow SP, Tracy SA, Estroff JA, Parad RB, Castro-Aragon IM, Fujii AM, *et al.* A role for abdominal ultrasound in discriminating suspected necrotizing enterocolitis in congenital heart disease patients. Pediatr Surg Int 2022;38:225-33.
17. Burge KY, Gunasekaran A, Makoni MM, Mir AM, Burkhart HM, Chaaban H. Clinical characteristics and potential pathogenesis of cardiac necrotizing enterocolitis in neonates with congenital heart disease: A narrative review. J Clin Med 2022;11:3987.
18. Giannone PJ, Luce WA, Nankervis CA, Hoffman TM, Wold LE. Necrotizing enterocolitis in neonates with congenital heart disease. Life Sci 2008;82:341-7.
19. Hackam DJ, Sodhi CP. Toll-like receptor-mediated intestinal inflammatory imbalance in the pathogenesis of necrotizing enterocolitis. Cell Mol Gastroenterol Hepatol 2018;6:229-38.e1.
20. Gomart A, Vallée A, Lecarpentier Y. Necrotizing enterocolitis: LPS/TLR4-induced crosstalk between canonical TGF-β/Wnt/β-catenin pathways and PPARγ. Front Pediatr 2021;9:713344.
21. Swanson L, Katkar GD, Tam J, Pranadinata RF, Chareddy Y, Coates J, *et al.* TLR4 signaling and macrophage inflammatory responses are dampened by GIV/Girdin. Proc Natl Acad Sci U S A 2020;117:26895-906.
22. Molteni M, Gemma S, Rossetti C. The role of toll-like receptor 4 in infectious and noninfectious inflammation. Mediators Inflamm 2016;2016:6978936.
23. Sodhi CP, Neal MD, Siggers R, Sho S, Ma C, Branca MF, *et al.* Intestinal epithelial toll-like receptor 4 regulates goblet cell development and is required for necrotizing enterocolitis in mice. Gastroenterology 2012;143:708-18.e5.
24. Leaphart CL, Cavallo J, Gribar SC, Cetin S, Li J, Branca MF, *et al.* A critical role for TLR4 in the pathogenesis of necrotizing enterocolitis by modulating intestinal injury and repair. J Immunol 2007;179:4808-20.
25. Xiong X, Bao Z, Mi Y, Wang X, Zhu J. Melatonin alleviates neonatal necrotizing enterocolitis by repressing the activation of the NLRP3 inflammasome. Gastroenterol Res Pract 2022;2022:6920577.
26. Cuna A, George L, Sampath V. Genetic predisposition to necrotizing enterocolitis in premature infants: Current knowledge, challenges, and future directions. Semin Fetal Neonatal Med 2018;23:387-93.
27. Jilling T, Ambalavanan N, Cotten CM, Martin CA, Maheshwari A, Schibler K, *et al.* Surgical necrotizing enterocolitis in extremely premature neonates is associated with genetic variations in an intergenic region of chromosome 8. Pediatr Res 2018;83:943-53.

28. Cai X, Golubkova A, Hunter CJ. Advances in our understanding of the molecular pathogenesis of necrotizing enterocolitis. BMC Pediatr 2022;22:225.

29. Szpecht D, Neumann-Klimasińska N, Błaszczyński M, Seremak-Mrozikiewicz A, Kurzawińska G, Cygan D, *et al*. Candidate gene analysis in pathogenesis of surgically and non-surgically treated necrotizing enterocolitis in preterm infants. Mol Cell Biochem 2018;439:53-63.

30. Fawley J, Cuna A, Menden HL, McElroy S, Umar S, Welak SR, *et al*. Single-immunoglobulin interleukin-1-related receptor regulates vulnerability to TLR4-mediated necrotizing enterocolitis in a mouse model. Pediatr Res 2018;83:164-74.

31. Sampath V, Menden H, Helbling D, Li K, Gastonguay A, Ramchandran R, *et al*. SIGIRR genetic variants in premature infants with necrotizing enterocolitis. Pediatrics 2015;135:e1530-4.

32. Walsh MC, Kliegman RM. Necrotizing enterocolitis: Treatment based on staging criteria. Pediatr Clin North Am 1986;33:179-201.

33. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics 2012;13:134.

34. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, *et al*. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. Genet Med 2015;17:405-24.

35. Kozyrev IA, Kotin NA, Averkin II, Ivanov AA, Latypov AA, Gordeev ML, *et al*. Modified technique for coarctation of aorta with hypoplastic distal aortic arch. J Card Surg 2021;36:2063-9.

36. Steri M, Idda ML, Whalen MB, Orrù V. Genetic variants in mRNA untranslated regions. Wiley Interdiscip Rev RNA 2018;9:e1474.

37. Cunningham F, Allen JE, Allen J, Alvarez-Jarreta J, Amode MR, Armean IM, *et al*. Ensembl 2022. Nucleic Acids Res 2022;50:D988-95.

38. Stanzick KJ, Li Y, Schlosser P, Gorski M, Wuttke M, Thomas LF, *et al*. Discovery and prioritization of variants and genes for kidney function in >1.2 million individuals. Nat Commun 2021;12:4350.

39. Richardson TG, Sanderson E, Palmer TM, Ala-Korpela M, Ference BA, Davey Smith G, *et al*. Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: A multivariable mendelian randomisation analysis. PLoS Med 2020;17:e1003062.

40. Khuseyinova N, Koenig W. Apolipoprotein A-I and risk for cardiovascular diseases. Curr Atheroscler Rep 2006;8:365-73.

41. Guo J, Rackham OJ, Sandholm N, He B, Österholm AM, Valo E, *et al*. Whole-genome sequencing of Finnish type 1 diabetic siblings discordant for kidney disease reveals DNA variants associated with diabetic nephropathy. J Am Soc Nephrol 2020;31:309-23.

42. Lau PE, Cruz SM, Ocampo EC, Nuthakki S, Style CC, Lee TC, *et al*. Necrotizing enterocolitis in patients with congenital heart disease: A single center experience. J Pediatr Surg 2018;53:914-7.

43. ElHassan NO, Tang X, Gossett J, Zakaria D, Ross A, Kona SK, *et al*. Necrotizing enterocolitis in infants with hypoplastic left heart syndrome following stage 1 palliation or heart transplant. Pediatr Cardiol 2018;39:774-85.

44. Lopez NL, Gowda C, Backes CH, Nandi D, Miller-Tate H, Fichtner S, *et al*. Differences in midterm outcomes in infants with hypoplastic left heart syndrome diagnosed with necrotizing enterocolitis: NPCQIC database analysis. Congenit Heart Dis 2018;13:512-8.

45. Luce WA, Schwartz RM, Beauseau W, Giannone PJ, Boettner BL, Cheatham JP, *et al*. Necrotizing enterocolitis in neonates undergoing the hybrid approach to complex congenital heart disease. Pediatr Crit Care Med 2011;12:46-51.

46. Fisher JG, Bairdain S, Sparks EA, Khan FA, Archer JM, Kenny M, *et al*. Serious congenital heart disease and necrotizing enterocolitis in very low birth weight neonates. J Am Coll Surg 2015;220:1018-26.e14.

47. Jeffries HE, Wells WJ, Starnes VA, Wetzel RC, Moromisato DY. Gastrointestinal morbidity after Norwood palliation for hypoplastic left heart syndrome. Ann Thorac Surg 2006;81:982-7.

48. Schuchardt EL, Kaufman J, Lucas B, Tiernan K, Lujan SO, Barrett C. Suspected necrotising enterocolitis after surgery for CHD: An opportunity to improve practice and outcomes. Cardiol Young 2018;28:639-46.

Supplemental Table 1: Designed primer nucleotide sequences

Name	Sequence (5'–>3')	PCR product size
SIGIRR_ex1_For	AGAACCACCAACTGCCCG	684
SIGIRR_ex1_Rev	CACATCACATACACACAGCCC	
SIGIRR_ex2_For	CGTGTGAGGGGCTCAAAGAG	589
SIGIRR_ex2_Rev	GTGCCCACTCCTAGCATTCC	
SIGIRR_ex3_For	AAGTGCTAAGCCTGTCCCCA	591
SIGIRR_ex3_Rev	ACCCTAGACTTTGCTGACACC	
SIGIRR_ex4-5_For	TAAGGCCCAAGAACCACCC	663
SIGIRR_ex4-5_Rev	ACCCGCCCCGGACTTTAA	
SIGIRR_ex6_For	CTGGGGTTAAAGTCCGGGG	590
SIGIRR_ex6_Rev	CCGAAAGCACCACGATGAG	
SIGIRR_ex7-8_For	GGCTACAAGCTCTTCCTGGAC	747
SIGIRR_ex7-8_Rev	GCCCATTCACAAAGCGTGG	
SIGIRR_ex9_For	AGCCACGGAATAGCTGTCG	526
SIGIRR_ex9_Rev	CTCACCCCCTGCTGTGATG	
SIGIRR_ex10_For	TCGGTCTGCCTGGGAACTT	525
SIGIRR_ex10_Rev	GAAGCCGAATCCGAAACCTTC	

PCR: Polymerase chain reaction