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Lymphatic anomalies during lifetime in patients with Noonan syndrome: Retrospective cohort study

Jessie W. Swarts[1] | Lotte E. R. Kleimeier[1] | Erika K. S. M Leenders[2] | Tuula Rinne[3] | Willemijn M. Klein[4] | Jos M. T. Draaisma[1]

[1]Department of Pediatrics, Amalia Children's Hospital, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, Netherlands

[2]Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands

[3]Department of Human Genetics, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, Netherlands

[4]Department of Medical Imaging, Radboud University Medical Center, Nijmegen, Netherlands

Correspondence
Jos M.T. Draaisma, Department of Pediatrics, Amalia Children's Hospital, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, Netherlands.
Email: jos.draaisma@radboudumc.nl

Abstract

Noonan syndrome (NS) has been associated with an increased risk of lymphatic anomalies, with an estimated prevalence of 20%. The prevalence of lymphatic anomalies seems to differ between pathogenic variants. Therefore, this study aims to describe the clinical presentation, prevalence and genotype–phenotype correlations of lymphatic anomalies during life in patients with NS. This retrospective cohort study included patients ($n = 115$) who were clinically and genetically diagnosed with NS and visited the Noonan expertise Center of the Radboud University Medical Center between January 2015 and March 2021. Data on lymphatic anomalies during lifetime were obtained from medical records. Lymphatic anomalies most often presented as an increased nuchal translucency, chylothorax and/or lymphedema. Prenatal lymphatic anomalies increased the risk of lymphatic anomalies during infancy (OR 4.9, 95% CI 1.7–14.6). The lifetime prevalence of lymphatic anomalies was 37%. Genotype–phenotype correlations showed an especially high prevalence of lymphatic anomalies during infancy and childhood in patients with a pathogenic *SOS2* variant ($p = 0.03$ and $p < 0.01$, respectively). This study shows that patients with NS have a high predisposition for developing lymphatic anomalies during life. Especially patients with prenatal lymphatic anomalies have an increased risk of lymphatic anomalies during infancy. Genotype–phenotype correlations were found in pathogenic variants in *SOS2*.

KEYWORDS
lymphatic anomalies, Noonan syndrome, postnatal, prenatal

1 | INTRODUCTION

Noonan syndrome (NS) is a predominantly autosomal-dominant genetic disorder, characterized by facial dysmorphism, congenital cardiac defects, and growth retardation (Tajan, Paccoud, et al., 2018; Tartaglia et al., 2011). The prevalence of NS is estimated at 1–5 per 10,000 live births (https://www.orpha.net). NS is part of the RASopathy family in which the Ras-mitogen-activated protein kinase (Ras/MAPK) pathway is dysregulated (Tajan, Paccoud, et al., 2018). Pathogenic variants in multiple elements of the pathway have been linked to NS, of which *PTPN11* (protein SHP-2) (50%) (Tartaglia

Jessie W. Swarts, Lotte E.R. Kleimeier, and Erika Leenders should be considered joint first author.

et al., 2011) *SOS1* (10%–18%) (Kouz et al., 2016; Tartaglia et al., 2011), *RAF1* (5%–15%) (Kouz et al., 2016; Tartaglia et al., 2011), and *RIT1* (5%) (Kouz et al., 2016) are the most common. At least 10 different genes have been linked to NS so far (Grant et al., 2018).

The Ras/MAPK pathway plays an important role in ERK signaling, which is suggested to play a key role in the development of the lymphatic system (Yamamoto et al., 2015). Pathogenic variants in genes in the Ras/MAPK pathway are associated with an abnormal development of the lymphatic system (de Mooij et al., 2011; Deng et al., 2013). Malformations in the lymphatic system can lead to a wide range of lymphatic anomalies. Enlarged jugular lymphatic sacs have been associated with an increased nuchal translucency (NT), often observed during prenatal ultrasounds (Haak et al., 2002). Other prenatal presentations of lymphatic anomalies are persistent nuchal fold (earlier used term cystic hygroma), hydrops fetalis, ascites, chylothorax, and chylopericardium (Croonen et al., 2013; Myers et al., 2014). Postnatal lymphatic anomalies have been reported in NS, most often presented as lymphedema (Joyce et al., 2016; Smpokou et al., 2012). Lymphatic anomalies such as chylothorax, ascites, chylopericardium, protein losing enteropathy and pulmonary lymphangiectasia have also been described (Biko et al., 2019; Hatemi et al., 2010; Joyce et al., 2016).

The estimated prevalence of lymphatic anomalies in NS patients is about 20% (Roberts et al., 2013; Romano et al., 2010). However, the expectation is that it occurs far more often (Myers et al., 2014). Both prenatal lymphatic anomalies as well as those presenting later in life seem to occur more often in patients with a pathogenic variant in *RIT1* or *SOS2* (Kouz et al., 2016; Lissewski et al., 2020; Sleutjes et al., 2022; Yaoita et al., 2016). No other genotype–phenotype correlations have been found (Ko et al., 2008; Smpokou et al., 2012; Zenker et al., 2004), and further elucidation is needed (Baldassarre et al., 2011; Milosavljevic et al., 2016; Wang et al., 2020). So far, only small cohort and case studies investigated the clinical presentation of lymphatic anomalies in NS patients, mainly focusing on prenatal lymphatic anomalies. A few cohort studies investigated the occurrence of lymphatic anomalies later in life, of which most only included lymphedema (Biko et al., 2019; Kouz et al., 2016; Schubbert et al., 2006; Shaw et al., 2007; Smpokou et al., 2012). However, the occurrence of lymphatic anomalies during lifetime is still not completely understood, and predictive factors remain unknown. Therefore, this study aims to provide an overview of the clinical presentation and prevalence of lymphatic anomalies in fetuses, infants, children, and adults with NS. In addition, genotype–phenotype correlations will be investigated.

2 | METHODS

2.1 | Editorial policies and ethical considerations

This study has been approved by the Medical Ethics Committee at Radboud University Medical Center Nijmegen (file number 2020-6852). Informed consent was acquired from all parents and, when appropriate, patients aged 12 years or older.

2.2 | Patient population

This retrospective cohort study selected patients from the Radboud University Medical Center in the Netherlands. Patients were eligible if they were clinically and genetically diagnosed with NS and visited the Noonan expertise Center of the Radboud University Medical Center between January 2015 and March 2021. All information regarding patients' characteristics, lymphatic anomalies, and genetic findings were extracted during a visit to the Noonan expertise center and documented in Epic by a pediatrician and/or clinical geneticist. Data was extracted from the Epic electronic medical record system (Epic Systems, Verona, WI, USA), and collected in a Castor-based database.

2.3 | Study variables

Clinical data, pre and postnatally and results from genetic test were assessed via medical records.

Patients with NS caused by a (likely) pathogenic variant in *BRAF* (NM_00443334), *KRAS* (NM_033360), *LZTR1* (NM_006767), *PTPN11* (NM_002834), *RAF1* (NM_002880), *RIT1* (NM_001256821), *RRAS2* (NM_012250), *SOS1* (NM_005633) and *SOS2* (NM_006939) variants were included. Additional information was collected for pathogenic variants in *PTPN11* on the predicted functional impact on SHP-2 function, consisting of A/I switching, A/I switching and catalysis, A/I switching and specificity, A/I switching and/or catalysis, SH2 pY-binding, SHP2 orientation or mobility or others (Tartaglia et al., 2006).

Pediatricians or clinical geneticists reported on prenatal ultrasounds findings. These prenatal ultrasound findings were used to collect data on prenatal lymphatic anomalies, consisting of an increased NT (NT >3.5 mm), ascites, persisted NT, chylothorax, chylopericardium, and hydrops fetalis. Information on postnatal lymphatic anomalies occurring as an infant (0–1 year), child (1–18 years), or adult (≥18 years), were obtained from medical files. Medical files were reviewed from birth until March 2021. Postnatal lymphatic anomalies included lymphedema of the upper or lower extremities, genital lymphedema, protein losing enteropathy, chylothorax, chylopericardium, pulmonary lymphangiectasia, and intestinal lymphangiectasia. Ascites, chylothorax, and chylopericardium were defined as either the presence of abnormal fluid accumulation on imaging techniques or confirmation by fluid analysis. Protein losing enteropathy was defined as an increased amount of intestinal alpha-1-antitrypsin clearance. The number of included patients for each stage of life will differ, because patients were only included until the stage of life related to the age at which they last visited the NS expertise center. Prenatal and postnatal lymphatic anomalies were combined in a variable for lifetime lymphatic anomalies. To consider the lymphatic anomalies in the frame of the NS phenotype, also due to the hypothesis that cardiovascular malformations might be due to compression by lymphatic obstruction (Witt et al., 1987), the correlation was analyzed between prenatal and postnatal (infant, child, and adult) lymphatic anomalies with the presence of congenital heart disease. This was also done for lifetime occurrence of chylothorax and lymphedema.

TABLE 1 Patients with *PTPN11* variants in current study (*n* = 71)

Gene	Nucleotide change	Amino acid change	Functional domain	Number of patients (%)	Interpretation	ClinVar review status	ClinVar accession
PTPN11				71 (60.2)			
	c.5C > T	p.(Thr2Ile)	A/I switching	2	Pathogenic/Likely pathogenic	2	VCV000013349
	c.124A > G	p.(Thr42Ala)	SH2 pY binding	3	Pathogenic	2	VCV000040482
	c.172A > G	p.(Asn58Asp)	A/I switching	3	Pathogenic	2	VCV000040487
	c.174C > G	p.(Asn58Lys)	A/I switching	1	Pathogenic/Likely pathogenic	2	VCV000040489
	c.179G > C	p.(Gly60Ala)	A/I switching	1	Pathogenic	2	VCV000040493
	c.181G > A	p.(Asp61Asn)	A/I switching	6	Pathogenic	2	VCV000040495
	c.182A > G	p.(Asp61Gly)	A/I switching	1	Pathogenic	2	VCV000013330
	c.186_188del	p.(Tyr63del)	A/I switching	1	Likely pathogenic	4	VCV000981579
	c.188A > G	p.(Tyr63Cys)	A/I switching	4	Pathogenic	1	VCV000013333
	c.205G > C	p.(Glu69Gln)	A/I switching	1	Pathogenic	1	VCV000040498
	c.214G > T	p.(Ala72Ser)	A/I switching	4	Pathogenic	2	VCV000013324
	c.218C > T	p.(Thr73Ile)	A/I switching	1	Pathogenic	3	VCV000013334
	c.236A > G	p.(Gln79Arg)	A/I switching	2	Pathogenic	2	VCV000013340
	c.317A > C	p.(Asp106Ala)	SH2 orientation or mobility	1	Pathogenic	2	VCV000040506
	c.328G > A	p.(Glu110Lys)	SH2 orientation or mobility	1	Pathogenic/Likely pathogenic	2	VCV000040507
	c.417G > C	p.(Glu139Asp)	SH2 pY-binding	7	Pathogenic	1	VCV000040513
	c.767A > G	p.(Gln256Arg)	A/I switching	2	Pathogenic	2	VCV000040518
	c.844A > G	p.(Ile282Val)	A/I switching and catalysis	2	Pathogenic	2	VCV000040525
	c.853 T > C	p.(Phe285Leu)	A/I switching and/or catalysis	1	Pathogenic	2	VCV000040528
	c.854 T > C	p.(Phe285Ser)	A/I switching and/or catalysis	1	Pathogenic	2	VCV000013335
	c.922A > G	p.(Asn308Asp)	A/I switching and/or catalysis	8	Pathogenic	1	VCV000013326
	c.923A > G	p.(Asn308Ser)	A/I switching and/or catalysis	2	Pathogenic	2	VCV000013327
	c.1471C > T	p.(Pro491Ser)	A/I switching and/or catalysis	1	Pathogenic	2	VCV000040550
	c.1492C > T	p.(Arg498Trp)	A/I switching and/or catalysis	2	Pathogenic	2	VCV000040553
	c.1507G > A	p.(Gly503Arg)	A/I switching and specificity	1	Pathogenic	2	VCV000040559
	c.1507G > C	p.(Gly503Arg)	A/I switching and specificity	3	Pathogenic	2	VCV000040558
	c.1508G > A	p.(Gly503Glu)	A/I switching and specificity	1	Pathogenic/Likely pathogenic	2	VCV000040561
	c.1510A > G	p.(Met504Val)	A/I switching and/or catalysis	7	Pathogenic	1	VCV000040562
	c.1529A > G	p.(Gln510Arg)	A/I switching and catalysis	1	Pathogenic/Likely pathogenic	2	VCV000013345

Note: Values are given in absolute number (percentage). Percentages may not add up to 100% due to rounding. ClinVar Review status: The interpretation of these variants is based on "ClinVar Review status:
1 = reviewed by expert panel, 2 = criteria provided, multiple submitters, no conflicts, 3 = conflicts about the interpretation in ClinVar, 4 = the variant was submitted only once or without interpretation, or
5 = the variant was absent in ClinVar" ACMG guideline was applied for variants without interpretation in ClinVar.

TABLE 2 Patients with variants in other genes than *PTPN11* in current study (n = 47)

Gene	Nucleotide change	Amino acid change	Number of patients (%)	Interpretation	ClinVar review status	ClinVar accession
SOS1			18 (15.3)			
	c.137A > T	p.(Gln46Leu)	1	Uncertain significance	5	n.a.
	c.305C > G	p.(Pro102Arg)	3	Likely pathogenic	2	VCV000477721
	c.508A > G	p.(Lys170Glu)	1	Pathogenic	1	VCV000040651
	c.797C > A	p.(Thr266Lys)	1	Pathogenic	2	VCV000012869
	c.806 T > C	p.(Met269Thr)	1	Pathogenic	1	VCV000040662
	c.1009 T > C	p.(Tyr337His)	1	Likely pathogenic	5	n.a.
	c.1642A > C	p.(Ser548Arg)	3	Pathogenic	1	VCV000040678
	c.1654A > G	p.(Arg552Gly)	3	Pathogenic	1	VCV000012871
	c.1656G > T	p.(Arg552Ser)	1	Pathogenic	1	VCV000040684
	c.2104 T > C	p.(Tyr702His)	1	Pathogenic	2	VCV000040696
	c.2207 T > G	p.(Ile736Arg)	1	Pathogenic	2	VCV000521933
	c.3134C > G	p.(Pro1045Arg)	1	Likely pathogenic	4	VCV000932923
KRAS			7 (5.9)			
	c.40G > A	p.(Val14Ile)	2	Pathogenic	1	VCV000012589
	c.53C > T	p.(Ala18Val)	1	Pathogenic	5	n.a.
	c.194_195ins21	p.(Ser65delins8)	1	Pathogenic	5	n.a.
	c.214A > C	p.(Met72Leu)	1	Likely pathogenic	4	VCV001016209
	c.478G > A	p.(Val160Met)	2	Uncertain significance	4	VCV000496492
LZTR1			7 (5.9)			
	c.848G > A	p.(Arg283Gln)	5	Pathogenic	3	VCV000561716
	c.1687G > C	p.(Glu563Gln)	compound heterozygous 1	Likely pathogenic	4	VCV001184933
	c.2090G > A	p.(Arg697Gln)		Likely pathogenic	3	VCV001206143
	c.2090G > A	p.(Arg697Gln)	compound heterozygous 1	Likely pathogenic	3	VCV001206143
	c.2407-2A > G			Likely pathogenic	3	VCV000809337
RAF1			6 (5.1)			
	c.766A > G	p.(Arg256Gly)	1	Pathogenic/Likely pathogenic	2	VCV000044631
	c.770C > T	p.(Ser257Leu)	2	Pathogenic	1	VCV000013957
	c.781C > T	p.(Pro261Ser)	1	Pathogenic	1	VCV000013958
	c.788 T > G	p.(Val263Gly)	1	Likely pathogenic	1	VCV000040607
	c.1457A > G	p.(Asp486Gly)	1	Pathogenic/Likely pathogenic	2	VCV000040618
SOS2			4 (3.4)			
	c.800 T > A	p.(Met267Lys)	2	Pathogenic	2	VCV000209092

(Continues)

TABLE 2 (Continued)

Gene	Nucleotide change	Amino acid change	Number of patients (%)	Interpretation	ClinVar review status	ClinVar accession
	c.800 T > C	p.(Met267Thr)	2	Pathogenic	2	VCV000373114
RIT1			3 (2.5)			
	c.118A > G	p.(Lys40Glu)	1	Pathogenic	5	n.a.
	c.170C > G	p.(Ala74Gly)	1	Pathogenic	2	VCV000060506
	c.280G > A	p.(Ala77Thr)	1	Pathogenic/Likely pathogenic	2	VCV000183403
BRAF			1 (0.8)			
	c.722C > T	p.(Thr241Met)		Pathogenic/Likely pathogenic	2	VCV000029805
RRAS2			1 (0.8)			
	c.208G > A	p.(Ala70Thr)	1	Pathogenic	4	VCV000626912

Note: Values are given in absolute number (percentage). Percentages may not add up to 100% due to rounding. ClinVar Review status: The interpretation of these variants is based on "ClinVar Review status: 1 = reviewed by expert panel, 2 = criteria provided, multiple submitters, no conflicts, 3 = conflicts about the interpretation in ClinVar, 4 = the variant was submitted only once or without interpretation, or 5 = the variant was absent in ClinVar" ACMG guideline was applied for variants without interpretation in ClinVar.

2.4 | Statistical analysis

The prevalence of lymphatic anomalies was calculated using descriptive statistics, and presented as the number of observations and as a percentage. The occurrence of lymphatic anomalies was investigated per stage of life (prenatally, infancy, childhood, or adulthood). The analyses were stratified by gene. Additional analyses were performed on the occurrence of lymphatic anomalies in patients with a pathogenic variant in *PTPN11* stratified by the predicted functional impact on SHP2 (Tartaglia et al., 2006), and on the correlation of lymphatic anomalies with congenital heart disease. Differences between groups were analyzed using fisher's exact test. Binary logistic regression analyses were used to analyze the association between prenatal and postnatal lymphatic anomalies, and presented as odds ratio (OR) with corresponding 95% confidence interval (CI). A *p* value below 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY).

3 | RESULTS

3.1 | Patient characteristics

A total of 118 patients clinically and genetically diagnosed with NS were seen between January 2015 and March 2021. The study population consisted of 2 infants, 83 children (<18 years) and 33 adults (≥18 years), of 61 males and 57 females, with a median age of 11 years (IQR: 5–20). Most patients had a pathogenic variant in the *PTPN11* gene (60.2%), followed by *SOS1* (15.3%), *KRAS* (5.9%), *LZTR1* (5.9%), and *RAF1* (5.1%). The gene distribution of the cohort can be found in Tables 1 and 2 and Table A1. Variants were classified according to the Clinvar and ACMG criteria (Gelb et al., 2018; Landrum et al., 2018; Richards et al., 2015). After re-classification, three cases were excluded from further analysis due to uncertain significance. However, they are still included in descriptive Table 2 and Table A1. There were four parent–child cases and five sib-sib cases (see also Table A1). Of these cases only one child had a prenatal lymphatic anomaly and transient lymphedema. The parent had no symptoms of lymphatic anomalies.

3.2 | Prevalence of lymphatic anomalies

The lifetime prevalence of lymphatic anomalies in the total cohort was 37%. Prenatal lymphatic anomalies were observed in 18 of 92 (20%) patients from which prenatal data were present, and postnatal lymphatic anomalies in 35 of 115 (30%) patients. The most prevalent prenatal lymphatic abnormalities were an increased NT and chylothorax with 9% and 6%, respectively. During infancy, lymphedema of the extremities (19%) and chylothorax (7%) were the most prevalent lymphatic anomalies. In children and adults, the highest prevalence was found for lymphedema of the extremities, 13% and 29% respectively.

TABLE 3 The clinical presentation of lymphatic anomalies in patients with NS with different genetic variants ($n = 118$)

	All patients[a] ($n = 115$)	PTPN11 ($n = 71$)	SOS1 ($n = 17$)	LZTR1 ($n = 7$)[b]	RAF1 ($n = 6$)	SOS2 ($n = 4$)	RIT1 ($n = 3$)	RRAS2 ($n = 1$)
Gender	61 m, 57 f	38 m, 33 f	9 m, 8 f	3 m, 4 f	4 m, 2 f	0 m, 4 f	1 m, 2 f	1 m, 0 f
Median age (IQR), years	11 (5–20)	11 (5–20)	8 (5–24)	12 (3–32)	13 (2–18)	14 (3–29)	(6, 14, 20)[c]	(1)[c]
Prenatal								
Ascites	1 (1)	—	—	1 (25)	—	—	—	—
Chylopericardium	1 (1)	1(2)	—	—	—	—	—	—
Cystic hygroma	1 (1)	—	1 (7)	—	—	—	—	—
Hydrops fetalis	4 (4)	3 (5)	1 (7)	—	—	—	—	—
Hydrothorax (chylothorax)	6 (6)	5 (9)	1 (7)	—	—	—	—	—
Increased NT[d]	9 (9)	6 (11)	—	1 (25)	1 (17)	1 (25)	—	—
Prenatal prevalence	18/92 (20)	12/57 (21)	2/13 (15)	2/4 (50)	1/6 (17)	1/4 (25)	0/3 (0)	0/1 (0)
Infant								
Chylopericardium	1(1)	—	—	—	1(1)	—	—	—
Chylothorax	8 (7)	4 (6)	1 (6)	1 (14)	1 (17)	—	1 (33)	—
Intestinal lymphangiectasia	—	—	—	—	—	—	—	—
Lymphedema extremities	22 (19)	8 (12)	3 (17)	3 (43)	3 (50)	3 (75)	1 (33)	1 (100)
Lymphedema genitals	3 (3)	1 (1)	—	—	—	—	1 (33)	1 (100)
Protein losing enteropathy	—	—	—	—	—	—	—	—
Pulmo. lymphangiectasia	—	—	—	—	—	—	—	—
Infancy prevalence	27/113 (24)	13/69 (19)	3/17 (18)	3/7 (43)	4/6 (67)	3/4 (75)[e]	1/3 (33)	1/1 (100)
Child								
Chylopericardium	—	—	—	—	—	—	—	—
Chylothorax	4 (4)	2 (3)	—	—	1 (20)	—	1 (33)	—
Intestinal lymphangiectasia	—	—	—	—	—	—	—	—
Lymphedema extremities	14 (13)	7 (10)	1 (7)	1 (14)	1 (20)	3 (100)	1 (33)	—
Lymphedema genitals	2 (2)	1 (2)	—	—	—	—	1 (33)	—
Protein losing enteropathy	—	—	—	—	—	—	—	—
Pulmo. lymphangiectasia	2 (2)	2 (3)	—	—	—	—	—	—
Childhood prevalence	16/106 (15)	8/68 (12)	1/14 (7)	1/7 (14)	2/5 (40)	3/3 (100)[e]	1/3 (33)	0/0 (0)
Adult								
Chylopericardium	—	—	—	—	—	—	—	—
Chylothorax	3 (10)	2 (11)	—	—	—	—	1 (100)	—
Intestinal lymphangiectasia	2 (6)	1 (5)	1 (13)	—	—	—	—	—
Lymphedema extremities	9 (29)	4 (21)	3 (43)	—	—	1 (100)	1 (100)	—
Lymphedema genitals	2 (7)	1 (5)	—	—	—	—	1 (100)	—

(Continues)

TABLE 3 (Continued)

	All patients[a] (n = 115)	PTPN11 (n = 71)	SOS1 (n = 17)	LZTR1 (n = 7)[b]	RAF1 (n = 6)	SOS2 (n = 4)	RIT1 (n = 3)	RRAS2 (n = 1)
Protein losing enteropathy	1 (3)	1 (5)	—	—	—	—	—	—
Pulmo. lymphangiectasia	1 (3)	1 (5)	—	—	—	—	—	—
Adulthood prevalence	12/31 (39)	7/19 (37)	3/7 (43)	0/2 (0)	0/1 (0)	1/1 (100)	1/1 (100)	0/0 (0)
Postnatal prevalence	35/115 (30)	19/71 (27)	5/17 (29)	3/7 (43)	3/6 (50)	3/4 (75)	1/3 (33)	1/1 (100)
Lifetime prevalence	43/115 (37)	25/71 (35)	6/17 (35)	3/7 (43)	4/6 (67)	3/4 (75)	1/3 (33)	1/1 (100)

Note: Values are given in absolute number (percentage) or absolute number/total (percentage).

Abbreviations: f, female; IQR, interquartile range; m, male; NT, nuchal translucency.

[a]Noonan syndrome with pathogenic *BRAF, KRAS, LZTR1, PTPN11, RAF1, RIT1, RRAS2, SOS1 and SOS2* variants.

[b]Two patients with a compound heterozygous variant.

[c]Age of each patient specified.

[d]Defined as a NT above 3.5 millimeters.

[e]PTPN11 versus other genes, $p < 0.05$ by fisher's exact test.

TABLE 4 Associations between prenatal lymphatic anomalies and lymphatic anomalies during infancy and childhood in patients with Noonan syndrome

	Postnatal lymphatic anomalies							
	Infant (n = 92)				Child (n = 84)			
	N	Yes	No	Odds ratio (95% CI)	N	Yes	No	Odds ratio (95% CI)
Prenatal lymphatic anomalies								
No	74	15	59	Reference	69	10	59	Reference
Yes	18	10	8	**4.9 (1.7–14.6)**	15	4	11	2.1 (0.6–8.1)

Note: Significant results are represented as bold numbers.

Abbreviations: CI, confidence interval; N, number.

No lymphatic anomalies were found in patients with a pathogenic variant in *KRAS or BRAF*. The clinical presentation of lymphatic anomalies in fetuses, infants, children, and adults with NS are shown in Table 3.

3.3 | Correlation between prenatal and postnatal lymphatic phenotype

Prenatal lymphatic anomalies were associated with the occurrence of lymphatic anomalies during infancy (OR 4.9, 95% CI 1.7–14.6; Table 4). No association was found between prenatal and childhood lymphatic anomalies (OR 2.1, 95% CI 0.6–8.1). Table 5 shows the clinical and genetical findings of patients who presented with prenatal lymphatic anomalies. An increased NT was observed in nine patients, of those 5 (56%) suffered from lymphedema as infant. Of the six patients with a chylothorax prenatally, 4 (67%) were diagnosed with a chylothorax neonatally. One of the patients with a chylothorax and hydrops fetalis prenatally, died 2 days after birth. The one patient with prenatal developed ascites suffered postnatally from lymphedema and a chylothorax. Table 6 gives an overview of patients with postnatal lymphatic anomalies, but without known prenatal lymphatic anomalies.

Intensive conservative treatment of lymphedema was necessary in five out of 19 patients with lymphedema during childhood or adulthood (Damstra, Halk, & Dutch Working Group on, 2017). This resulted in significant decrease of lymphedema in two patients. Two other patients were treated with a MEK-inhibitor because of therapy resistant complaints and underlying central conducting Lymphatic anomaly, which was diagnosed by dynamic contrast-enhanced MR lymphangiography. In addition, one patient, also diagnosed with an underlying central conducting lymphatic anomaly by dynamic contrast-enhanced MR lymphangiography, was treated with operative interventions.

3.4 | Genotype–phenotype correlation

Pathogenic variants in *PTPN11* were found in 71 patients, of those 25 (35%) presented with lymphatic anomalies during lifetime (Table 3). An increased NT and chylothorax were the most prevalent prenatal lymphatic anomalies, detected in 11% and 9% of the patients, respectively. During adulthood, *PTPN11* patients also presented with protein losing enteropathy (5%) and chylothorax (11%) (Table 3).

TABLE 5 Demographics, lymphatic anomalies, and genetic findings of patients with Noonan syndrome with prenatal lymphatic problems (n = 18)

| General information | | | Lymphatic anomalies | | | | Genetic findings | | | Additional information |
| | | | | Postnatal | | | | | | |
ID	Sex	Age last observation	Prenatal	Infancy	Child	Adult	Affected gene	Nucleotide change	Amino acid change	Congenital heart disease
7-2	F	3 y	AC	CT, LO	–	NA	LZTR1	c.848G > A	p.(Arg283Gln)	HCM
11	F	12 y	NT	LO	–	NA	LZTR1	c.2090G > A; c.2407-2A > G	p.(Arg697Gln); p.(?)	HCM, ASD, VSD
71	F	12 y	CT	–	–	NA	PTPN11	c.1510A > G	p.(Met504Val)	PVS
22	F	8 y	NT	LO	LO	NA	PTPN11	c.181G > A	p.(Asp61Asn)	PVS
30	M	19 y	NT	–	–	–	PTPN11	c.188A > G	p.(Tyr63Cys)	
31	M	21 m	NT	–	NA	NA	PTPN11	c.188A > G	p.(Tyr63Cys)	
49	F	3 y	CP, HF	–	–	NA	PTPN11	c.417G > C	p.(Glu139Asp)	PVS
48	F	16 y	NT	LO	–	NA	PTPN11	c.417G > C	p.(Glu139Asp)	
47	F	18 y	CT	CT	LO	NA	PTPN11	c.417G > C	p.(Glu139Asp)	ASD, VSD
13	F	2 m	CT, HF	–	NA	NA	PTPN11	c.5C > T	p.(Thr2Ile)	
53	M	9 y	CT	CT	–	NA	PTPN11	C.844A > G	p.(Ile282Val)	PVS, ASD
29	F	3 y	CT, HF	CT	–	NA	PTPN11	c.186_188del	p.(Tyr63del)	PVS
14	F	23 y	NT	LO	–	–	PTPN11	c.124A > G	p.(Thr42Ala)	AVSD
35	F	29 y	NT	–	–	–	PTPN11	c.214G > T	p.(Ala72Ser)	PVS, ASD
81	M	11 m	NT	CP			RAF1	c.770C > T	p.(Ser257Leu)	HCM
92	M	0 y[a]	CT, HF	CT, LO	NA	NA	SOS1	c.508A > G	p.(Lys170Glu)	
93	F	5 y	CH	–	–	NA	SOS1	c.797C > A	p.(Thr266Lys)	VSD, PDA
107	F	17 y	NT	LO	LO	NA	SOS2	c.800 T > A	p.(Met267Lys)	PVS, ASD

Abbreviations: AC, ascites; ASD, atrial septal defect; AVSD, atrial ventricular septal defect; CH, cystic hygroma; CP, chylopericardium; CT, chylothorax; F, female; HCM, hypertrophic cardiomyopathy; LO, lymphedema of upper or lower extremities; M, male; m, months; NA, not available; NT, increased nuchal translucency; PVS, pulmonary valve stenosis; PDA, patent ductus arteriosus; PVS, pulmonary valve stenosis; VSD, ventricular septal defect; y, years.

[a]Died.

TABLE 6 Demographics, lymphatic anomalies, and genetic findings of patients with Noonan syndrome without known prenatal lymphatic problems (n = 25)

General information				Lymphatic anomalies			Genetic findings			Additional information
				Postnatal						
ID	Sex	Age first observation	Age last observation (y)	Infancy	Child	Adult	Affected gene	Nucleotide change	Amino acid change	Congenital heart disease
9	M	PN	8	LO	LO	NA	LZTR1	c.848G > A	p.(Arg283Gln)	
16	F	PN	17	LO	LO, PL	NA	PTPN11	c.124A > G	p.(Thr42Ala)	ASD
20	F	0 y	32	–	–	LO	PTPN11	c.174C > G	p.(Asn58Lys)	
24	F	0 y	32	LO, LG	LO, LG	LO, LG	PTPN11	c.181G > A	p.(Asp61Asn)	PVS, ASD
23	F	0 y	55	–	–	CT, LO	PTPN11	c.181G > A	p.(Asp61Asn)	
25	M	PN	5	LO	–	NA	PTPN11	c.181G > A	p.(Asp61Asn)	ASD
39	M	0 y	22	LO, LG	LO, LG	LO, LG, PLE	PTPN11	c.c.218C > T	p.(Thr73Ile)	PVS, ASD
32	M	PN	7	LO	–	NA	PTPN11	c.188A > G	P.(Tyr63Cys)	
40	M	68 y	73	NA	–	CT	PTPN11	c.236A > G	p.(Gln79Arg)	
44	M	0 y	47	NA	–	LO	PTPN11	c.417G > C	p.(Glu139Asp)	PVS, ASD
55	M	PN	3	CT	CT, PL	NA	PTPN11	c.854 T > C	p.(Phe285Ser)	HCM
58	M	PN	15	LO	–	NA	PTPN11	c.922A > G	p.(Asn308Asp)	
63	F	0 y	51	NA	NA	LO	PTPN11	c.923A > G	p.(Asn308Ser)	
70	M	7 y	17	–	LO	NA	PTPN11	c.1508G > A	p.(Gly503Glu)	PVS, ASD
79	M	PN	23	LO	–	–	RAF1	c.766A > G	p.(Arg256Gly)	HCM
80	F	PN	16	–	CT	NA	RAF1	c.770C > T	p.(Ser257Leu)	PVS, HCM
83	M	PN	3	LO	LO	NA	RAF1	c.788 T > G	p.(Val263Gly)	PVS, HCM
87	M	PN	20	–	CT, LO, LG	CT, LO, LG	RIT1	c.280G > A	p.(Ala77Thr)	PVS, ASD
88	M	PN	2	LO, LG	NA	NA	RRAS2	c.208G > A	p.(Ala70Thr)	VSD
90	F	0 y	41	NA	NA	LO	SOS1	c.305C > G	p.(Pro102Arg)	
96	M	0 y	63	NA	NA	LO	SOS1	c.1642A > C	p.(Ser548Arg)	
100	M	PN	3	LO	NA	NA	SOS1	c.1654A > G	p.(Arg552Gly)	PVS
102	M	PN	33	LO	LO	LO[a]	SOS1	c.1656G > T	p.(Arg552Ser)	PVS, HCM, ASD
106	F	PN	12	LO	LO	NA	SOS2	c.800 T > A	p.(Met267Lys)	PVS
108	F	PN	33	LO	LO	LO	SOS2	c.800 T > C	p.(Met267Thr)	HCM

Abbreviations: ASD, atrial septal defect; AVSD, atrial ventricular septal defect; CT, chylothorax; F, female; HCM, hypertrophic cardiomyopathy; LG, lymphedema of the genitals; LO, lymphedema of upper or lower extremities; M, male; m, months; NA, not available; PDA, patent ductus arteriosus; PL, pulmonary lymphangiectasia; PN, prenatal; PVS, pulmonary valve stenosis; VSD, ventricular septal defect; Y, years.
[a]Until the age of 26 years.

TABLE 7 Lymphatic anomalies in patients with a pathogenic variant in *PTPN11* in different functional domains (*n* = 61)

Occurrence	A/I switching (*n* = 29)	A/I switching and/or catalysis (*n* = 22)	SH2 pY-binding (*n* = 10)
Prenatal	6/25 (24)	1/18 (6)	4/8 (50)[a]
Postnatal	9/29 (31)	3/22 (14)	5/10 (50)[a]
Infancy	5/29 (17)	2/21 (10)	4/10 (40)
Childhood	2/24 (8)	2/20 (10)	3/8 (38)
Adulthood	5/10 (50)	1/5 (20)	2/4 (50)
Lifetime	13/29 (45)[a]	4/22 (18)	6/10 (60)[a]

Note: Values are given in absolute number/total (percentage).
[a]A/I switching and/or catalysis versus other functional domains, *p* < 0.05 by fisher's exact test.

Table 7 shows the prevalence of lymphatic anomalies in different pathogenic variants in *PTPN11* stratified by the predicted functional impact on SHP2-function. Most of the pathogenic variants were located in or close to the N-SH2/PTP-interacting surface perturbing the equilibrium between active and inactive protein conformation (group I, A/I switching, *n* = 28), followed by variants that were also affecting catalysis (group IV, A/I switching and/or catalysis, *n* = 23), and variants involved in SH2 pY-binding (group V, *n* = 10). Due to the small numbers of patients in the other functional domains (group II: A/I switching and catalysis *n* = 3; III: A/I switching and specificity *n* = 5; VI: SH2 orientation or mobility *n* = 2) (Tartaglia et al., 2006), analysis was performed only for the three largest groups. Patients with a predicted effect on SH2 pY-binding had significantly more prenatal, postnatal and lifetime lymphatic anomalies than patients with a predicted effect on A/I switching and/or catalysis (respectively *p* = 0.02, *p* = 0.04 and *p* = 0.04). Patients with variants with a predicted effect on A/I switching had significantly more lifetime lymphatic anomalies than patients with A/I switching and/or catalysis (*p* = 0.04).

Of the 17 patients with a pathogenic *SOS1* variant, six (35%) presented with lymphatic anomalies (Table 3). Prenatal lymphatic anomalies occurred in two of 13 (15%) patients with available data on prenatal development, and postnatally in five of 17 (29%) patients. As can be seen in Table 5, the prenatal ultrasound of patient 92 showed a chylothorax and hydrops fetalis. Neonatally this infant boy was diagnosed with massive lymphedema and a chylothorax. He died 2 days after birth due to the severity of those lymphatic anomalies. One patient (patient 102), without prenatal lymphatic anomalies, suffered from lymphedema of the lower extremities during infancy, childhood and adulthood. The lymphedema disappeared after cardiac surgery.

Lymphatic anomalies during lifetime were observed in four of six (67%) patients with a pathogenic *RAF1* variant (Table 3). Patient 81 presented with increased NT prenatally and presented with pericardial fluids postnatally (Table 5). Patient 80 with a pathogenic variant in *RAF1*, and without information of prenatal ultrasounds, developed a chylothorax after cardiac surgery.

Two of seven patients with variants in *LZTR1* had an autosomal recessive form of NS due to compound heterozygous variants. One patient, with variants c.2090G > A p.(Arg697Gln) and c.2407-2A > G p.(?) had an increased NT and lymphatic anomalies during infancy. The other five patients, with an autosomal dominant form of NS, all had the same variant c.848G > A; p.Arg283Gln. Two of these five patients had lymphatic anomalies during lifetime.

Patients with a pathogenic variant in *SOS2* had a lifetime prevalence of lymphatic anomalies of 75% (Table 3). Patients with a pathogenic variant in *SOS2* had a higher prevalence of lymphatic anomalies during infancy and childhood compared to patients with a pathogenic *PTPN11* variant (*p* = 0.03 and *p* < 0.01, respectively). Postnatal lymphatic anomalies only presented as lymphedema of the extremities, which occurred in three of the four *SOS2* patients during childhood.

Three patients had pathogenic variants in *RIT1*, of those one (33%) developed lymphatic anomalies during lifetime. This patient had a chylothorax, lymphedema of the extremities and genitals during infancy, childhood, and adulthood.

One patient had a pathogenic variant in *RRAS2*. This patient had lymphedema of the extremities and genitals as an infant.

3.5 | Correlation with the presence of congenital heart disease

There was no correlation between prenatal lymphatic presentations (by ultrasound) and the presence of a congenital heart disease (*p* = 0.50), nor was there a correlation between postnatal lymphatic anomalies and the presence of a congenital heart disease (*p* = 1.00), the lifetime occurrence of chylothorax and congenital heart disease (*p* = 0.45) and lymphedema and congenital heart disease (*p* = 0.43).

4 | DISCUSSION

This study investigated the clinical presentation of lymphatic anomalies in patients with NS. The lifetime prevalence of lymphatic anomalies was 37% in the 115 patients included in our study, which is higher compared to the commonly estimated prevalence of 20% (Roberts et al., 2013; Romano et al., 2010). Prenatal lymphatic anomalies occurred in 20% of all patients, which is lower than previously reported (Hakami et al., 2016; Li et al., 2019; Myers et al., 2014). Myers et al. identified prenatal lymphatic anomalies by ultrasound in 54/102 (53%) patients with NS (Myers et al., 2014). However, their study included patients from medical literature, mostly case reports and case series, instead of an original cohort. A systematic review by Sleutjes et al. reported on the prevalence of specific prenatal lymphatic anomalies, and found a prevalence of 33% for cystic hygroma, 25% for an increased NT, and 19% for pleural effusions (Sleutjes et al., 2022). Whereas prenatal lymphatic anomalies in this cohort most often presented as increased NT and chylothorax. Nevertheless, clinical features such as low-set angulated ears, webbed neck, and

wide spaced nipples are thought to be a result of prenatal lymphatic obstruction (Allanson, 2007). These clinical features are highly prevalent in NS, since 90% of patients have low-set angulated ears (Romano et al., 2010). Consequently, it is expected that prenatal lymphatic anomalies occur in the majority of patients with NS. However, not all prenatal lymphatic anomalies might be detectable by prenatal ultrasounds, which could have led to an inevitable underestimation of the prenatal prevalence within our cohort.

Prenatal ultrasound anomalies were suggested to be associated with a more severe postnatal phenotype (Baldassarre et al., 2011). In line with these findings, this cohort study found an association between the occurrence of prenatal lymphatic anomalies detected by ultrasounds and lymphatic anomalies during infancy. Four of the six (67%) patients with chylothorax prenatally, had a chylothorax during infancy. Of those with an increased NT prenatally, 56% suffered from lymphedema as an infant. Furthermore, patients from other medical centers who died shortly after birth were not referred to our expertise center. Table A2 portrays the prenatal, and if possible postnatal, lymphatic findings of these patients within our medical center who died shortly after birth and were not diagnosed with NS before death. Therefore, they were unable to visit our NS expertise center, and not included in this study. These data suggest the correlation between the occurrence of prenatal lymphatic anomalies including hydrops fetalis and chylothorax and severe postnatal lymphatic anomalies. More severe cases might not be included and therefore the actual numbers are expected to be even higher. Furthermore, no association was found between prenatal lymphatic anomalies and a lymphatic phenotype during childhood. In general, childhood lymphatic anomalies were less common. Nevertheless, the prevalence of lymphatic anomalies increased again during adulthood. Joyce et al. suggested a progressive course of lymphatic anomalies, with the onset of chylothorax and/or protein losing enteropathy (Joyce et al., 2016). A late onset of protein losing enteropathy was also found by Wang et al. (Wang et al., 2020). Since patients in the present cohort are still susceptible as they become older, the prevalence of lymphatic anomalies during adulthood is expected to become higher. Overall, the prevalence of postnatal lymphatic anomalies was 30%. The previously published study from Smpokou et al. reported that 49% of the NS patients suffered from postnatal lymphedema (Smpokou et al., 2012). Myers et al. even found a prevalence of 68% for neonatal lymphatic dysplasia (Myers et al., 2014). Nevertheless little is known on the exact prevalence of lymphatic anomalies during adulthood, and the prevalence of this cohort might become higher as our patients are still aging.

Lymphatic anomalies did not occur in association with all genes. Only patients with pathogenic variants in PTPN11, RAF1, RIT1, RRAS2, SOS1, SOS2, or LZTR1 genes had lymphatic anomalies during lifetime. However, some of these genes were represented only by a small number of cases. Therefore the prevalence of lymphatic anomalies in these genes should be interpreted with caution. A recently conducted systematic review by Sleutjes et al., showed that prenatal lymphatic anomalies occurred in patients with pathogenic variants in the PTPN11, SOS1, RAF1, KRAS, RIT1, and BRAF gene (Sleutjes et al., 2022). Prenatal lymphatic anomalies were not reported in

patients with a pathogenic SOS2 variant, mostly since only one case of SOS2 was included. In addition, no articles were included on patients with a pathogenic variant in LZTR1. Prenatal lymphatic anomalies were recently also reported in patients with pathogenic KRAS, BRAF, NRAS and LZTR1 variants (Scott et al., 2021).

Furthermore, Sleutjes et al. reported postnatal lymphatic anomalies in patients with pathogenic variants in the PTPN11, SOS1, RIT1, KRAS and BRAF genes (Sleutjes et al., 2022). Often, the number of cases per involved genes is low due to a low-genetic prevalence. Therefore, results of several studies may need to be combined in order to gain more knowledge on the occurrence of lymphatic anomalies in low-prevalent genes.

The study of Pagnamenta et al. suggested a more severe lymphatic phenotype in patients with biallelic pathogenic variant in LZTR1, especially hydrops fetalis (Pagnamenta et al., 2019). In the current study, there were two patients with an autosomal recessive form of NS due to compound heterozygous variants. One patient had lymphedema of the lower extremities during infancy. There were five patients with an autosomal dominant form of NS, all with the same heterozygous pathogenic variant. Two patients had lymphedema of the lower extremities during infancy and/or childhood, with one patient also having a chylothorax in infancy. In total three of seven patients with LZTR1 variants showed symptoms of lymphatic disorder. There were no patients with hydrops fetalis. However, one of the patients that was not included in our study (Table A2), with a heterozygous variant in LZTR1, died soon after birth as a result of severe lymphatic complications including hydrops fetalis. These data suggests that there is a high risk of lymphatic anomalies during lifetime for patients with NS due to variants in LZTR1 in both the autosomal dominant and recessive form of NS. However, the number of patients with variants in LZTR1 in this study is small and future studies are needed to investigate this further.

Patients with a pathogenic variant in SOS2 had an especially high prevalence of lymphatic anomalies during infancy and childhood. Likewise, Lissewski et al. found a correlation between a pathogenic variant in SOS2 and lymphatic anomalies. Of note, this study included three patients also included in this cohort (Lissewski et al., 2020). Similar to the present study, almost all SOS2 patients described in literature developed lymphedema during childhood (Cordeddu et al., 2015; Ding et al., 2019; Lissewski et al., 2020). The prevalence of prenatal lymphatic anomalies in SOS2 patients was 29% in a previous study (Lissewski et al., 2020). In this cohort, 25% of the SOS2 patients had prenatal lymphatic anomalies. Furthermore, one out of three (33%) RIT1 patients had severe lymphatic anomalies during several stages of life. Previous studies reported a genotype–phenotype correlation of lymphatic anomalies in patients with a pathogenic variant in RIT1 (Kouz et al., 2016; Sleutjes et al., 2022). One of the included patients had a pathogenic variant in RRAS2. This patient suffered from lymphedema of the extremities and genitals during infancy. Little is known about the phenotype of RRAS2 patients. Therefore, further studies and case-reports are needed to unravel the phenotype and prevalence of lymphatic anomalies in NS patients due to pathogenic variants in RRAS2.

The genotype–phenotype correlations of pathogenic *PTPN11* variants and the predicted effect on SHP-2 function showed a higher prenatal, postnatal and overall lifetime prevalence of lymphatic anomalies for variants affecting the SH2 pY-binding than for variants affecting A/I switching and/or catalysis. Patients with a *PTPN11* variant affecting A/I switching had a higher lifetime prevalence of lymphatic anomalies than patients with a *PTPN11* variant affecting the A/I switching and/or catalysis. No other study has investigated this correlation before. Further studies are required to confirm and further investigate this finding.

There was no correlation between the presence of prenatal lymphatic anomalies (by ultrasound) and the presence of a congenital heart disease. This is in line with earlier publications (Baldassarre et al., 2011; Gaudineau et al., 2013), and does not support the hypothesis that cardiovascular malformations might be due to compression by lymphatic obstruction (Witt et al., 1987).

So far, the treatment of lymphatic anomalies depends on the clinical presentation, and is mainly conservative in nature. Identifying the causal genes, and intervening in the responsible pathway may give therapeutic opportunities. Recently, the use of a MEK-inhibitor has shown to remodel the central conducting lymphatic system in a patient with NS due to a pathogenic variant in *SOS1* (Dori et al., 2020). The treatment led to the resolution of the clinical symptoms of the lymphatic anomalies. Therefore, inhibition of the Ras-MAPK pathway seems to be a promising new therapy for patients with NS complicated by an underlying central conducting lymphatic anomaly.

To limit bias, patients were only included if they physically visited the NS expertise centrum between 2015–2021 and were examined by an expert pediatrician and clinical geneticist with specific expertise on NS. Patients who were diagnosed with NS intrauterine between 2015–2020 were also included, which explains the inclusion of patient 16 (Table 5). It is, however, important to note that prenatal ultrasound results were either extracted from available documentation, or (parents of) patients were asked if they had been informed of any abnormalities. Therefore, prenatal abnormalities could have been misclassified as having no prenatal lymphatic anomalies, which inevitably leads to bias. Patients who died within the first couple of weeks after birth were not included, because they mostly did not visit the NS expertise center. One patient however was diagnosed with NS prenatally, which made early visits possible. Another limitation is that patients with severe lymphatic anomalies could have died prenatally, or before genetic testing was performed. However, especially those patients might have a higher prevalence of lymphatic anomalies. Due to this, lymphatic anomalies might be underreported. A recent study shows that the use of Ultra-Rapid Exome Sequencing is feasible in critically ill pediatric patients (Australian Genomics Health Alliance Acute Care et al., 2020). This new method could overcome the above-mentioned limitations in the future. The distribution of genes among our study population is, in general, similar to those previously reported (Table A3) (Bertola et al., 2020; El Bouchikhi et al., 2016; Kouz et al., 2016; Romano et al., 2010; Shoji et al., 2019; Tajan, Pernin-Grandjean, et al., 2018). Nevertheless, within this study

population there are less patients with pathogenic variant in *LZTR1* and *RIT1* genes, and more with a pathogenic variant in *SOS2*. Therefore, the statements made regarding these genes should be interpreted with caution.

To our knowledge, this is the first large-scale study providing an overview of the clinical presentation and prevalence of lymphatic anomalies in patients with NS according to the stage of life.

In conclusion, patients with NS have a high predisposition for developing lymphatic anomalies during life. Especially patients with prenatal lymphatic anomalies had an increased risk of lymphatic anomalies during infancy. Prenatal lymphatic anomalies most often presented as increased NT and chylothorax, whereas postnatal lymphatic anomalies most often presented as lymphedema. In addition, pathogenic variants in the *SOS2* gene especially show a high prevalence.

ORCID

Jessie W. Swarts https://orcid.org/0000-0003-4985-5782
Lotte E. R. Kleimeier https://orcid.org/0000-0002-0553-7064
Jos M. T. Draaisma https://orcid.org/0000-0003-4829-6173

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APPENDIX

TABLE A1 Overview genetic findings per patient (n = 118)

Family ID	Sex	Gene	Nucleotide change	Amino acid change	Transcript	Inheritance	Age first observation	Age last observation	Lifetime lymphatic anomalies
1	F	BRAF	c.722C > T	p.(Thr241Met)	NM_004333.4	De novo	PN	8 y	No
2-1	F	KRAS	c.40G > A	p.(Val14Ile)	NM_033360.2	De novo (sibling 2–2)	1y	15 y	No
2-2	M	KRAS	c.40G > A	p.(Val14Ile)	NM_033360.2	De novo (sibling 2–1)	2y	14 y	No
3	M	KRAS	c.53C > T	p.(Ala18Val)	NM_033360.2	De novo	PN	9 y	No
4	F	KRAS	c.194_195ins21	p.(Ser65delins8)	NM_033360.2	De novo	PN	9 y	No
5	M	KRAS	c.214A > C	p.(Met72Leu)	NM_004985.4	Unknown	PN	18 y	No
6-1	M	KRAS	c.478G > A	p.(Val160Met)	NM_033360.3	Maternal (affected) (sibling 6–2)	PN	15 y	No
6-2	F	KRAS	c.478G > A	p.(Val160Met)	NM_033360.3	Maternal (affected) (sibling 6–1)	PN	13 y	No
7-1	M	LZTR1	c.848G > A	p.(Arg283Gln)	NM_006767.3	De novo (parent 7–2)	0y	39 y	No
7-2	F	LZTR1	c.848G > A	p.(Arg283Gln)	NM_006767.3	Paternal (affected) (child 7–1)	PN	3 y	Yes
8-1	F	LZTR1	c.848G > A	p.(Arg283Gln)	NM_006767.3	Paternal (affected) (parent 8–2)	0y	32 y	No
8-2	M	LZTR1	c.848G > A	p.(Arg283Gln)	NM_006767.3	Maternal (affected) (child 8–1)	PN	1 y	No
9	M	LZTR1	c.848G > A	p.(Arg283Gln)	NM_006767.3	Paternal (affected)	PN	8 y	Yes
10	F	LZTR1	c.1687G > C; c.2090G > A	p.(Glu563Gln); p.(Arg697Gln)	NM_006767.3	Paternal/maternal (not affected)	0y	27 y	No
11	F	LZTR1	c.2090G > A; c.2407-2A > G	p.Arg697Gln; p.(?)	NM_006767.3	Paternal/maternal (not affected)	PN	12 y	Yes
12	M	PTPN11	c.5C > T	p.(Thr2Ile)	NM_002834.3	Unknown	PN	12 y	No
13	F	PTPN11	c.5C > T	p.(Thr2Ile)	NM_002834.3	De novo	PN	2 m	Yes
14	F	PTPN11	c.124A > G	p.(Thr42Ala)	NM_002834.3	De novo	PN	23 y	Yes
15	M	PTPN11	c.124A > G	p.(Thr42Ala)	NM_002834.3	De novo	PN	14 y	No
16	F	PTPN11	c.124A > G	p.(Thr42Ala)	NM_002834.3	De novo	PN	17 y	Yes
17	F	PTPN11	c.172A > G	p.(Asn58Asp)	NM_002834.3	Maternal (affected)	PN	13 y	No
18	M	PTPN11	c.172A > G	p.(Asn58Asp)	NM_002834.3	De novo	PN	8 y	No
19	M	PTPN11	c.172A > G	p.(Asn58Asp)	NM_002834.3	Maternal (affected)	PN	7 y	No

TABLE A1 (Continued)

Family ID	Sex	Gene	Nucleotide change	Amino acid change	Transcript	Inheritance	Age first observation	Age last observation	Lifetime lymphatic anomalies
20	F	PTPN11	c.174C > G	p.(Asn58Lys)	NM_002834.3	De novo	0y	32 y	Yes
21	F	PTPN11	c.179G > C	p.(Gly60Ala)	NM_002834.3	De novo	PN	13 y	No
22	F	PTPN11	c.181G > A	p.(Asp61Asn)	NM_002834.3	De novo	PN	8 y	Yes
23	F	PTPN11	c.181G > A	p.(Asp61Asn)	NM_002834.3	Unknown	0y	55 y	Yes
24	F	PTPN11	c.181G > A	p.(Asp61Asn)	NM_002834.3	De novo	0y	32 y	Yes
25	M	PTPN11	c.181G > A	p.(Asp61Asn)	NM_002834.3	De novo	PN	5 y	Yes
26	F	PTPN11	c.181G > A	p.(Asp61Asn)	NM_002834.3	De novo	PN	2 y	No
27	F	PTPN11	c.181G > A	p.(Asp61Asn)	NM_002834.3	De novo	PN	18 y	No
28	F	PTPN11	c.182A > G	p.(Asp61Gly)	NM_002834.3	De novo	15y	35 y	No
29	F	PTPN11	c.186_188del	p.(Tyr63del)	NM_002834.3	De novo	PN	3 y	Yes
30	M	PTPN11	c.188A > G	p.(Tyr63Cys)	NM_002834.3	De novo	PN	19 y	Yes
31	M	PTPN11	c.188A > G	p.(Tyr63Cys)	NM_002834.3	Maternal (affected)	PN	8 y	No
32	M	PTPN11	c.188A > G	p.(Tyr63Cys)	NM_002834.3	Paternal (affected)	PN	7 y	Yes
33	M	PTPN11	c.188A > G	p.(Tyr63Cys)	NM_002834.3	Maternal (affected)	PN	21 m	Yes
34	M	PTPN11	c.205G > C	p.(Glu69Gln)	NM_002834.3	De novo	PN	11 y	No
35	F	PTPN11	c.214G > T	p.(Ala72Ser)	NM_002834.3	Unknown	PN	29 y	Yes
36	F	PTPN11	c.214G > T	p.(Ala72Ser)	NM_002834.3	De novo	PN	18 y	No
37	M	PTPN11	c.214G > T	p.(Ala72Ser)	NM_002834.3	Unknown	PN	7 y	No
38	F	PTPN11	c.214G > T	p.(Ala72Ser)	NM_002834.3	Unknown	PN	45 y	No
39	M	PTPN11	c.218C > T	p.(Thr73Ile)	NM_002834.3	De novo	0y	22 y	Yes
40	M	PTPN11	c.236A > G	p.(Gln79Arg)	NM_002834.3	Unknown	68y	73 y	Yes
41	F	PTPN11	c.236A > G	p.(Gln79Arg)	NM_002834.3	De novo	PN	9 y	No
42	F	PTPN11	c.317A > C	p.(Asp106Ala)	NM_002834.3	De novo	PN	11 y	No
43	M	PTPN11	c.328G > A	p.(Glu110Lys)	NM_002834.3	Paternal (affected)	PN	3 y	No
44	M	PTPN11	c.417G > C	p.(Glu139Asp)	NM_002834.3	Unknown	0y	47 y	Yes
45	M	PTPN11	c.417G > C	p.(Glu139Asp)	NM_002834.3	Unknown	12y	21 y	No
46	M	PTPN11	c.417G > C	p.(Glu139Asp)	NM_002834.3	De novo	0y	20 y	No
47	F	PTPN11	c.417G > C	p.(Glu139Asp)	NM_002834.3	De novo	PN	18 y	Yes
48	F	PTPN11	c.417G > C	p.(Glu139Asp)	NM_002834.3	De novo	PN	16 y	Yes
49	F	PTPN11	c.417G > C	p.(Glu139Asp)	NM_002834.3	De novo	PN	3 y	Yes
50	M	PTPN11	c.417G > C	p.(Glu139Asp)	NM_002834.3	De novo	PN	3 y	No
51-1	F	PTPN11	c.767A > G	p.(Gln256Arg)	NM_002834.3	Unknown (parent 51–2; affected)	PN	52 y	No

(Continues)

TABLE A1 (Continued)

Family ID	Sex	Gene	Nucleotide change	Amino acid change	Transcript	Inheritance	Age first observation	Age last observation	Lifetime lymphatic anomalies
51–2	M	PTPN11	c.767A > G	p.(Gln256Arg)	NM_002834.3	Maternal (affected) (child 51–1)	PN	21 y	No
52	F	PTPN11	c.844A > G	p.(Ile282Val)	NM_002834.3	De novo	PN	10 y	No
53	M	PTPN11	c.844A > G	p.(Ile282Val)	NM_002834.3	De novo	PN	9 y	Yes
54	F	PTPN11	c.853 T > C	p.(Phe285Leu)	NM_002834.3	De novo	PN	9 y	No
55	M	PTPN11	c.854 T > C	p.(Phe285Ser)	NM_002834.3	De novo	PN	3 y	Yes
56	F	PTPN11	c.922A > G	p.(Asn308Asp)	NM_002834.3	Maternal (affected)	0y	55 y	No
57	M	PTPN11	c.922A > G	p.(Asn308Asp)	NM_002834.3	No	PN	17 y	No
58	M	PTPN11	c.922A > G	p.(Asn308Asp)	NM_002834.3	Unknown	PN	15 y	Yes
59–1	M	PTPN11	c.922A > G	p.(Asn308Asp)	NM_002834.3	Paternal (affected) (sibling 59–2)	PN	7 y	No
59–2	M	PTPN11	c.922A > G	p.(Asn308Asp)	NM_002834.3	Paternal (affected) (sibling 59–1)	PN	5 y	No
60	M	PTPN11	c.922A > G	p.(Asn308Asp)	NM_002834.3	De novo	PN	6 y	No
61	F	PTPN11	c.922A > G	p.(Asn308Asp)	NM_002834.3	Unknown	PN	4 y	No
62	M	PTPN11	c.922A > G	p.(Asn308Asp)	NM_002834.3	Paternal (affected)	PN	4 y	No
63	F	PTPN11	c.923A > G	p.(Asn308Ser)	NM_002834.3	Unknown	0y	51 y	Yes
64	M	PTPN11	c.923A > G	p.(Asn308Ser)	NM_002834.3	De novo	PN	37 y	No
65	M	PTPN11	c.1471C > T	p.(Pro491Ser)	NM_002834.3	De novo	PN	9 y	No
66–1	M	PTPN11	c.1492C > T	p.(Arg498Trp)	NM_002834.3	De novo (parent 66–2; affected)	PN	34y	No
66–2	M	PTPN11	c.1492C > T	p.(Arg498Trp)	NM_002834.3	Paternal (affected) (child 66–1)	PN	7 y	No
67	M	PTPN11	c.1507G > A	p.(Gly503Arg)	NM_002834.4	Maternal (affected)	PN	5 y	No
68	M	PTPN11	c.1507G > C	p.(Gly503Arg)	NM_002834.3	De novo	PN	20 y	No
69–1	M	PTPN11	c.1507G > C	p.(Gly503Arg)	NM_002834.3	Maternal (affected) (twin 69–2)	PN	2 y	No
69–2	M	PTPN11	c.1507G > C	p.(Gly503Arg)	NM_002834.3	Maternal (affected) (twin 69–1)	PN	2 y	No
70	M	PTPN11	c.1508G > A	p.(Gly503Glu)	NM_002834.3	Maternal (affected)	7y	17 y	Yes
71	F	PTPN11	c.1510A > G	p.(Met504Val)	NM_002834.3	De novo	PN	12 y	Yes
72	F	PTPN11	c.1510A > G	p.(Met504Val)	NM_002834.3	Maternal (affected)	0 y	12 y	No
73	M	PTPN11	c.1510A > G	p.(Met504Val)	NM_002834.3	De novo	PN	11 y	No
74	M	PTPN11	c.1510A > G	p.(Met504Val)	NM_002834.3	De novo	0y	10 y	No
75	F	PTPN11	c.1510A > G	p.(Met504Val)	NM_002834.3	De novo	PN	9 y	No

TABLE A1 (Continued)

Family ID	Sex	Gene	Nucleotide change	Amino acid change	Transcript	Inheritance	Age first observation	Age last observation	Lifetime lymphatic anomalies
76	F	PTPN11	c.1510A > G	p.(Met504Val)	NM_002834.3	Unknown	PN	5 y	No
77	F	PTPN11	c.1510A > G	p.(Met504Val)	NM_002834.3	De novo	PN	1 y	No
78	F	PTPN11	c.1529A > G	p.(Gln510Arg)	NM_002834.3	De novo	PN	4 y	No
79	M	RAF1	c.766A > G	p.(Arg256Gly)	NM_002880.3	De novo	PN	23 y	Yes
80	F	RAF1	c.770C > T	p.(Ser257Leu)	NM_002880.3	De novo	PN	16 y	Yes
81	M	RAF1	c.770C > T	p.(Ser257Leu)	NM_002880.3	De novo	PN	11 m	Yes
82	M	RAF1	c.781C > T	p.(Pro261Ser)	NM_002880.3	De novo	PN	10 y	No
83	M	RAF1	c.788 T > G	p.(Val263Gly)	NM_002880.3	Unknown	PN	3 y	Yes
84	F	RAF1	c.1457A > G	p.(Asp486Gly)	NM_002880.3	De novo	PN	17 y	No
85	F	RIT1	c.118A > G	p.(Lys40Glu)	NM_001256821.1	De novo	PN	15 y	No
86	F	RIT1	c.170C > G	p.(Ala74Gly)	NM_001256821.1	De novo	PN	7 y	No
87	M	RIT1	c.280G > A	p.(Ala77Thr)	NM_001256821.1	Unknown	PN	20 y	Yes
88	M	RRAS2	c.208G > A	p.(Ala70Thr)	NM_012250.6	De novo	PN	2 y	Yes
89	M	SOS1	c.137A > T	p.(Gln46Leu)	NM_005633.3	Maternal (affected)	PN	16 y	No
90	F	SOS1	c.305C > G	p.(Pro102Arg)	NM_005633.3	Paternal (affected)	0y	41 y	Yes
91-1	F	SOS1	c.305C > G	p.(Pro102Arg)	NM_005633.3	Maternal (affected) (sibling 91–2)	PN	7 y	No
91-2	F	SOS1	c.305C > G	p.(Pro102Arg)	NM_005633.3	Maternal (affected) (sibling 91–1)	PN	5 y	No
92	M	SOS1	c.508A > G	p.(Lys170Glu)	NM_005633.3	De novo	PN	0	Yes
93	F	SOS1	c.797C > A	p.(Thr266Lys)	NM_005633.3	De novo	PN	5 y	Yes
94	M	SOS1	c.806 T > C	p.(Met269Thr)	NM_005633.3	De novo	PN	21 y	No
95	F	SOS1	c.1009 T > C	p.(Tyr337His)	NM_005633.3	Paternal (affected)	PN	10 y	No
96	M	SOS1	c.1642A > C	p.(Ser548Arg)	NM_005633.3	De novo	0y	63 y	Yes
97	F	SOS1	c.1642A > C	p.(Ser548Arg)	NM_005633.3	Maternal (affected)	10y	20 y	No
98	M	SOS1	c.1642A > C	p.(Ser548Arg)	NM_005633.3	Paternal (affected)	PN	15 y	No
99	F	SOS1	c.1654A > G	p.(Arg552Gly)	NM_005633.3	De novo	PN	5 y	No
100	M	SOS1	c.1654A > G	p.(Arg552Gly)	NM_005633.3	Unknown	PN	3 y	Yes
101	F	SOS1	c.1654A > G	p.(Arg552Gly)	NM_005633.3	De novo	PN	2 y	No
102	M	SOS1	c.1656G > T	p.(Arg552Ser)	NM_005633.3	De novo	PN	33 y	Yes
103	M	SOS1	c.2104 T > C	p.(Tyr702His)	NM_005633.3	Maternal (affected)	PN	5 y	No
104	M	SOS1	c.2207 T > G	p.(Ile736Arg)	NM_005633.3	Maternal (affected)	PN	1 y	No

(Continues)

TABLE A1 (Continued)

Family ID	Sex	Gene	Nucleotide change	Amino acid change	Transcript	Inheritance	Age first observation	Age last observation	Lifetime lymphatic anomalies
105	M	SOS1	c.3134C > G	p.(Pro1045Arg)	NM_005633.3	Maternal (affected)	36y	48 y	No
106	F	SOS2	c.800 T > A	p.(Met267Lys)	NM_006939.3	De novo	PN	12 y	Yes
107	F	SOS2	c.800 T > A	p.(Met267Lys)	NM_006939.3	De novo	PN	17 y	Yes
108	F	SOS2	c.800 T > C	p.(Met267Thr)	NM_006939.3	De novo	PN	33 y	Yes
109	F	SOS2	c.800 T > C	p.(Met267Thr)	NM_006939.3	Paternal (affected)	PN	2 y	No

Note: Transcript numbers: *BRAF* (NM_004433334), *KRAS* (NM_033360), *LZTR1* (NM_006767), *PTPN11* (NM_002834), *RAF1* (NM_002880), *RIT1* (NM_001256821), *RRAS2* (NM_012250), *SOS1* (NM_005633) and *SOS2* (NM_006939).

Abbreviations: F, female; M, male; m, months; y, years; PN, prenatal.

TABLE A2 Prenatal and postnatal lymphatic findings of three patients that have died shortly after birth

General information			Lymphatic anomalies		Genetic findings			Additional information
ID	M/F	Age at time of study	Prenatal	Postnatal	Affected gene	Nucleotide change	Amino acid change	
1	M	0 y 1 m	CT, HF	CT	*PTPN11*	c.922A > G	p.Asn308Asp	Died at 6 weeks after cardiac surgery
2	F	0 y 1 m	—	CT	*PTPN11*	c.124A > G	p.Thr42Ala	Died at 8 weeks due to chylothorax
3	F	0 y 0 m	HF, NT	CP, CT, LE	*LZTR1*	c.848G > A	p.Arg283.Gln	Died after birth due to severe circulatory and respiratory insufficiency

Abbreviations: CP, chylopericardium; CT, chylothorax; F, female; HF, hydrops fetalis; LE, lymphedema of the extremities; M: male; m: months; NT: increased nuchal translucency.

TABLE A3 Distribution of genes involved in patients with Noonan syndrome in the present cohort ($n = 118$) and in literature

	Number of patients (%)	Prevalence in literature	Reference
PTPN11	71 (60.2)	35%–56%	(Bertola et al., 2020; El Bouchikhi et al., 2016; Romano et al., 2010; Shoji et al., 2019; Tajan et al., 2018)
SOS1	18 (15.3)	10%–19%	(Bertola et al., 2020; El Bouchikhi et al., 2016; Kouz et al., 2016; Romano et al., 2010; Shoji et al., 2019; Tajan et al., 2018)
KRAS	7 (5.9)	2%–10%	(Bertola et al., 2020; El Bouchikhi et al., 2016; Romano et al., 2010; Shoji et al., 2019)
LZTR1	7 (5.9)	7%	(Bertola et al., 2020)
RAF1	6 (5.1)	5%–15%	(Bertola et al., 2020; El Bouchikhi et al., 2016; Kouz et al., 2016; Romano et al., 2010; Shoji et al., 2019; Tajan et al., 2018)
SOS2	4 (3.4)	1%	(Bertola et al., 2020)
RIT1	3 (2.5)	4%–8%	(Bertola et al., 2020; El Bouchikhi et al., 2016; Kouz et al., 2016; Shoji et al., 2019)
BRAF	1 (0.8)	2%–4%	(Bertola et al., 2020; El Bouchikhi et al., 2016; Romano et al., 2010; Shoji et al., 2019)
RRAS2	1 (0.8)	Unknown	

Note: Values are given in absolute number (percentage). Percentages may not add up to 100% due to rounding.