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ORIGINAL ARTICLE

Retrospective study of prenatal ultrasound findings in newborns with a Noonan spectrum disorder

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ABSTRACT

Objectives Noonan spectrum disorders (NSDs) occur in 1:1000–2500 live births. Currently, there are no guidelines for prenatal molecular genetic testing for NSDs. Recent studies recommend prenatal testing for NSDs when ultrasonography detects two or more associated abnormalities. A stronger association between ultrasound findings and NSDs would enable more informed prenatal genetic testing.

Methods A total of 212 newborns (0–12 weeks) with prenatal ultrasound findings and a clinical suspicion of a NSD were referred for molecular genetic testing. Of these, 159/212 newborns tested had a single ultrasound abnormality and 53/212 newborns had two or more. Testing was performed by either a microarray-based resequencing assay or next generation sequencing of RAS/MAPK pathway genes associated with NSDs. Prenatal ultrasound findings in positive and negative cases were compared.

Results A disease-causing variant was identified in 21.7% (46/212) of newborns tested. Of these positive cases, 67.4% (31/46) had only one ultrasound abnormality reported. The rate of detecting a disease-causing variant in cases with one ultrasound finding was 19.5% (31/159), which was not significantly different (p-value = 0.36) than that in cases with two or more ultrasound findings (28.3%; 15/53).

Conclusions Prenatal molecular testing for NSDs should be considered even in the presence of a single associated abnormal ultrasound finding. © 2016 John Wiley & Sons, Ltd..

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INTRODUCTION

Noonan spectrum disorders (NSDs), or RASopathies, are a group of autosomal dominant developmental conditions caused by hyperactivation of the Ras-mitogen-activated protein kinase (RAS-MAPK) pathway. With an incidence as high as 1:1000–2500 live births,[1] NSDs constitute one of the most common groups of clinically and genetically heterogeneous disorders encountered in clinical genetic practice. These include Noonan syndrome (NS), Noonan syndrome with multiple lentigines (NSML) also known as LEOPARD syndrome, Costello syndrome (CS), cardio-facio-cutaneous syndrome (CFCS), and Legius syndrome (LS).

NSDs exhibit numerous overlapping phenotypic features including short stature, cardiovascular defects (such as pulmonary valve stenosis and hypertrophic cardiomyopathy), cutaneous abnormalities, and characteristic facial features. Skeletal anomalies, hematological abnormalities, developmental delays, and intellectual disabilities can also be associated with NSDs.[2] NSDs are associated with a large number of genes in the RAS-MAPK pathway, including *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *HRAS*, *BRAF*, *MAP2K1* (*MEK1*), *MAP2K2* (*MEK2*), *NRAS*, *SHOC2*, *SPRED1*, *CBL*, and *RIT1*.[2,3]

NSDs may present prenatally with abnormal ultrasound findings, and despite the advances in ultrasonography, the detected abnormalities are non-specific and do not correlate with the severity of the postnatal phenotype.[4,5] Prenatal ultrasound findings associated with NSDs include cardiac anomaly, cystic hygroma, increased nuchal translucency (NT), hydrops, polyhydramnios, lymphatic dysplasia, relative macrocephaly, pleural and pericardial effusion, ascites, and renal anomaly.[4,6] However, these prenatal features could also be identified in fetuses with other conditions such as chromosomal rearrangements and aneuploidies.[7] After ruling out a chromosome anomaly, the detection rate of NSD in a fetus with NSD-associated ultrasound findings has been reported to be as high as 17%.[8]

A prenatal suspicion of NSDs may be confirmed using molecular genetic testing, although expense has traditionally discouraged such testing. To increase the detection rate of NSDs, studies have suggested molecular testing when ultrasound findings include increased NT or cystic hygroma in combination with one additional finding.[8,9] However, it is unknown how many fetuses with NSD would not qualify for diagnostic testing if it were limited to only fetuses with two or more ultrasound findings. Additional data on the association between ultrasound findings and NSDs may support a different reliance on molecular genetic testing, enabling more accurate genetic counseling and family planning. In the present study, we evaluated the prenatal ultrasound findings in 212 newborns with a clinical suspicion of NSD, who were sent for genetic testing, to determine if there was difference in the detection rate in those with one ultrasound finding versus two or more ultrasound findings.

MATERIALS AND METHODS

Two hundred and twelve newborns (0 day–12 weeks old) with a clinical diagnosis or suspicion of a NSD and previously noted ultrasound anomalies were referred to the Laboratory for Molecular Medicine (LMM) from September 2005 through August 2015 for molecular genetic testing. DNA from peripheral blood samples was extracted using PureGene Blood Core Kit B (Qiagen #1042606). Between September 2005 and April 2008, five DNA samples were tested by Sanger sequencing of one or more of the following genes: *PTPN11*, *SOS1*, *RAF1*, and *KRAS*. From January 2009 to January 2010, 31 DNA samples were tested for eight genes (*BRAF*, *HRAS*, *KRAS*, *MAP2K1* (*MEK1*), *MAP2K2* (*MEK2*), *PTPN11*, *RAF1*, and *SOS1*) using a microarray-based resequencing assay (Affymetrix GeneChip) as previously described.[10] Using this same method, *NRAS* and the recurrent variant in *SHOC2* (c.4A > G; p.Ser2Gly) were added to the chip for 73 and 81 DNA samples, respectively. Eighty-six DNA samples were tested between May 2012 and December 2014 using next generation sequencing (NGS) of 12 genes (*PTPN11*, *SOS1*, *RAF1*, *KRAS*, *HRAS*, *BRAF*, *MAP2K1* (*MEK1*), *MAP2K2* (*MEK2*), *NRAS*, *SPRED1*, *CBL*, and exon 02 of *SHOC2*) as previously described.[11] On January 2015, *RIT1* was added to the genes tested for nine DNA samples. Briefly, NGS analysis was performed by oligonucleotide hybridization-based DNA capture using Agilent SureSelect followed by sequencing of the coding regions and splice sites using the Illumina HiSeq2000 instrument (50-base paired end mode) or MiSeq-M01450 instrument (150-base paired end mode). Sequence reads were aligned to the human reference sequence (GRCh37) using Burrows-Wheeler Aligner, followed by variant calling using GATK (version 1.0.4705).[12] Sanger sequencing was used to confirm all clinically significant variants and fill in regions with insufficient coverage. Methods used for polymerase chain reaction and Sanger sequencing have been previously described.[13] Variants were classified as pathogenic, likely pathogenic, uncertain significance, likely benign, and benign, as previously described.[14] Variants of uncertain significance (VUS) were further subcategorized into three categories: VUS-favor pathogenic when there is a suspicion of a pathogenic role but insufficient evidence to classify the variant as likely pathogenic, VUS-favor benign when the evidence suggests the variant does not contribute to disease but is insufficient to classify it as likely benign, and VUS when there is a lack of or conflicting evidence. In this study, only pathogenic and likely pathogenic variants were considered positive results. Variants classified as likely benign or benign were not Sanger confirmed. Physician-reported prenatal ultrasound findings in cases positive or negative for disease-causing variants were compared. Two-tailed Fisher's exact test was used to compute statistical significance. This study was approved by the Partners HealthCare Institutional Review Board.

RESULTS

A total of 21.7% (46/212) newborns with a clinical suspicion of a NSD and anomalies detected by ultrasound were found to have a pathogenic or likely pathogenic variant in one of the assayed genes (Table 1). Of those who had positive genetic testing, 41 newborns were indicated as having NS, three with CFCS, one with NSML, and one with CS. However, because of the rapid change in the facial appearance of newborns with an NSD and overlap in phenotypes[15], the reported clinical diagnosis for a specific NSD may not be accurate. Pathogenic and likely pathogenic variants in *PTPN11* constituted the majority of the variants identified, with 21 unique variants detected in 31 newborns (67.4% of positive cases). The second most commonly affected gene was *SOS1* with different pathogenic variants identified in six cases (13% of positive case). No disease-causing variants were identified in *CBL*, *MAP2K2*, *SHOC2*, *RIT1*, or *SPRED1*. However, a very limited number of samples had *RIT1* tested. Nine newborns were found to have variants classified as VUS (Table 2) and, therefore, considered to have an inconclusive test result. Eight of these newborns were indicated as having NS and one as having CFCS.

Consistent with the variable expressivity seen in NSD-associated postnatal phenotypes, there was no genotype-prenatal phenotype correlation. Nine variants – seven in *PTPN11*, one in *SOS1*, and one in *RAF1* – were identified in multiple newborns, each with different ultrasound abnormalities (Table 1). For example, the c.923A > G (p.Asn308Ser) variant in *PTPN11* was detected in a newborn with polyhydramnios as well as in a newborn with a CHD.

The majority of positive cases had only one anomaly reported by prenatal ultrasound. Out of the 212 newborns tested, 159 had one ultrasound abnormality while 53 had two or more (Table 1 and Table S1). The rate of detecting a disease-causing variant in cases with one ultrasound finding was 19.5% (31/159), which was not significantly different (Fisher exact test, p-value = 0.36) compared to cases with two or more ultrasound findings (28.3%; 15/53). Of the 46 newborns with positive results, disease-causing variants were detected in 15 (32.6%) newborns with two or more ultrasound findings, while 31 (67.4%) had only one of the following ultrasound findings: CHD, cystic hygroma, increased NT, polyhydramnios, pleural effusion, or hydrops.

CHD was the most common abnormality detected by prenatal ultrasound in molecularly confirmed NSD newborns,

Table 1 **Pathogenic and likely pathogenic variants identified**

Gene	mRNA transcript	Newborn	Clinical suspicion	Ultrasound findings	cDNA change	Amino acid change	Class.	Ref.
PTPN11	NM_002834	1	NS	Cystic hygroma	c.172A > C	p.Asn58His	P	20
		2	NS	CHD	c.172A > G	p.Asn58Asp	P	21
		3	NS	Cystic hygroma, hydrops, polyhydramnios and pleural effusion	c.179G > C	p.Gly60Ala	P	22
		4	NS	Cystic hygroma	c.181G > A	p.Asp61Asn	P	22
		5	NS	Pleural effusion	c.181G > A	p.Asp61Asn	P	22
		6	NS	Cystic hygroma and CHD	c.182A > G	p.Asp61Gly	P	23
		7	NS	CHD	c.182A > G	p.Asp61Gly	P	23
		8	NS	Pleural effusion	c.182A > G	p.Asp61Gly	P	23
		9	NS	Cystic hygroma and hydrops	c.211T > C	p.Phe71Leu	LP	24
		10	NS	Cystic hygroma, pleural effusion and pericardial effusion	c.214G > C	p.Ala72Pro	LP	17
		11, 12	NS	CHD	c.214G > T	p.Ala72Ser	P	23
		13	NS	Cystic hygroma	c.215C > G	p.Ala72Gly	P	23
		14	NS	CHD and increased NT	c.215C > G	p.Ala72Gly	P	23
		15	NS	Cystic hygroma, polyhydramnios and pleural effusion	c.217_218delinsCT	p.Thr73Leu	P	25
		16	NS	Hydrops	c.218C > T	p.Thr73Ile	P	22
		17	NS	Hydrops	c.417G > C	p.Glu139Asp	P	22
		18	NSML	CHD	c.836A > G	p.Tyr279Cys	P	22
		19	NS	CHD	c.853T > C	p.Phe285Leu	P	22
		20	NS	Cystic hygroma and CHD	c.853T > C	p.Phe285Leu	P	22
		21	NS	Cystic hygroma	c.854T > C	p.Phe285Ser	P	22
		22, 23	NS	CHD	c.922A > G	p.Asn308Asp	P	23
		24	NS	Cystic hygroma and CHD	c.922A > G	p.Asn308Asp	P	23
		25	CFCS	CHD	c.923A > G	p.Asn308Ser	P	22
		26	NS	Polyhydramnios	c.923A > G	p.Asn308Ser	P	22
		27	NS	Cystic hygroma and CHD	c.1381G > A	p.Ala461Thr	P	26
		28	NS	CHD	c.1381G > A	p.Ala461Thr	P	26
		29	NS	CHD	c.1492C > T	p.Arg498Trp	P	27
		30	NS	Cystic hygroma and increased NT	c.1505C > T	p.Ser502Leu	P	28
		31	NS	Cystic hygroma	c.1507G > C	p.Gly503Arg	P	29
SOS1	NM_005633	32	NS	Polyhydramnios	c.508A > G	p.Lys170Glu	P	30
		33	NS	Polyhydramnios and short femurs	c.1294T > C	p.Trp432Arg	P	31
		34	NS	Cystic hygroma	c.1642A > C	p.Ser548Arg	P	31
		35	NS	Cystic hygroma and increased NT	c.1655G > A	p.Arg552Lys	P	31
		36	NS	Hydrops and pyelectasis	c.1655G > A	p.Arg552Lys	P	31
		37	NS	Increased NT	c.2536G > A	p.Glu846Lys	P	31
RAF1	NM_002880	38	NS	Cystic hygroma and CHD	c.770C > T	p.Ser257Leu	P	32
		39	NS	Increased NT	c.770C > T	p.Ser257Leu	P	32
		40	CFCS	Cystic hygroma, CHD and increased NT	c.770C > T	p.Ser257Leu	P	32
KRAS	NM_004985	41	NS	Increased NT	c.173C > T	p.Thr58Ile	P	33
		42	NS	Increased NT	c.178G > C	p.Gly60Arg	P	34
BRAF	NM_004333	43	NS	Cystic hygroma	c.1447A > C	p.Lys483Gln	LP[a]	Novel
HRAS	NM_005343	44	CS	Cystic hygroma	c.175_176delinsCT	p.Ala59Leu	LP[a]	Novel
MAP2K1	NM_002755	45	CFCS	CHD	c.383G > T	p.Gly128Val	LP	35
NRAS	NM_002524	46	NS	Cystic hygroma	c.34G > A	p.Gly12Ser	LP	36

NS, Noonan syndrome; NSML,Noonan syndrome with multiple lentigines; CS, Costello syndrome; CFCS, cardio-facio-cutaneous syndrome; CHD, congenital heart defect; NT, nuchal translucency; Class, classification; P, pathogenic; LP, likely pathogenic; Ref, original reference for the variant.
[a]apparently *de novo* occurrence in affected individual (paternity not confirmed).

Table 2 **Variants of unknown significance identified**

Gene	mRNA Transcript	Newborn	Clinical suspicion	Ultrasound findings	cDNA change	Amino acid change	Class.	Ref.
CBL	NM_005188	1	NS	CHD	c.1380_1382dupTGA	p.Asp460dup	VUS-FB	Novel
		2	NS	CHD	c.2589C > G	p.Asn863Lys	VUS-FB	Novel
MAP2K1	NM_002755	3	CFCS	Hydrops	c.875C > G	p.Thr292Ser	VUS	Novel
		4	NS	CHD, pleural effusion, ascites	c.1039G > A	p.Ala347Thr	VUS-FB	8
MAP2K2	NM_030662	5	NS	CHD	c.391G > A	p.Val131Met	VUS	Novel
RAF1	NM_002880	6	NS	Severe edema	c.776C > A	p.Ser259Tyr	VUS-FP	Novel
		7	NS	CHD and polyhydramnios	c.935 T > C	p.Val312Ala	VUS-FB	8
SOS1	NM_005633	8	NS	IUGR	c.3347-1G > A	—	VUS	37
		9	NS	CHD	c.512 T > G	p.Val171Gly	VUS-FP	Novel

NSl, Noonan syndrome; CFCS, cardio-facio-cutaneous syndrome; CHD, congenital heart defect; IUGR, intrauterine growth restriction; Class, classification; VUS-FP, variant of uncertain significance—favor pathogenic; VUS, variant of uncertain significance; VUS-FB, variant of uncertain significance—favor benign; Ref., original reference for the variant.

followed by cystic hygroma and then increased NT (Table 3; Figure 1). There was no difference in the type of CHD identified; however, the number of positive cases with a CHD was limited. These findings were also commonly detected in negative and inconclusive cases both in isolation and in combination with other ultrasound abnormalities (Table S1). Overall, no single ultrasound abnormality or combination of ultrasound findings was predictive for NSD (Table 3), which is consistent with previous studies.[4,9]

DISCUSSION

This study identified a 21.7% (46/212) positive detection rate for NSD in newborns with previous prenatal ultrasound findings, which is consistent but slightly higher than a prior reported detection rate of 17.3% in prenatal samples.[8] This slight increase is likely caused by differences in the cohorts and number of genes tested, including an increase from 4 to 13 genes in our current study.

The *PTPN11* and *SOS1* genes were the most commonly affected genes among the 46 positive newborns. While previous reports have also found a high incidence of disease-causing *PTPN11* variants in prenatal samples with NSD-associated ultrasound findings[8,16,17], no studies, to the best of our knowledge, have identified disease-causing *SOS1* variants in prenatal samples with NSD-associated ultrasound findings.[8,9,17,18] Pergament and colleagues[18] identified a variant in *SOS1* (p.Pro655Leu) in two fetuses with increased NT; however, this variant is now known to be a common variant in the general population with a frequency of 1.2% in the European chromosomes.[19] The low detection rate of disease-causing *SOS1* variants in affected fetuses has been ascribed to *SOS1* variants possibly causing milder

Table 3 **Ultrasound findings in newborns**

Reported ultrasound findings	Newborns with positive genetic testing		Newborns with negative or inconclusive genetic testing		*p*-Value*
	number	%	number	%	
CHD[a]	12	26.1	65	39.2	0.31
Cystic hygroma	9	19.6	19	11.5	0.23
Increased NT	4	8.7	8	4.8	0.47
Polyhydramnios	2	4.3	6	3.6	0.69
Pleural effusion	2	4.3	3	1.8	0.31
Hydrops	2	4.3	14	8.4	0.53
1 other[b] U/S finding	0	0	13	7.8	0.31
CHD and cystic hygroma	5	10.9	13	7.8	0.56
2+ other U/S findings	10	21.8	25	15.1	0.39
Total	46	—	166	—	—

CHD, congenital heart defect; NT, nuchal translucency; U/S, ultrasound.

*Fisher exact test.

[a]See Table S2 for specific heart defect.

[b]See Table S1 for specific finding.

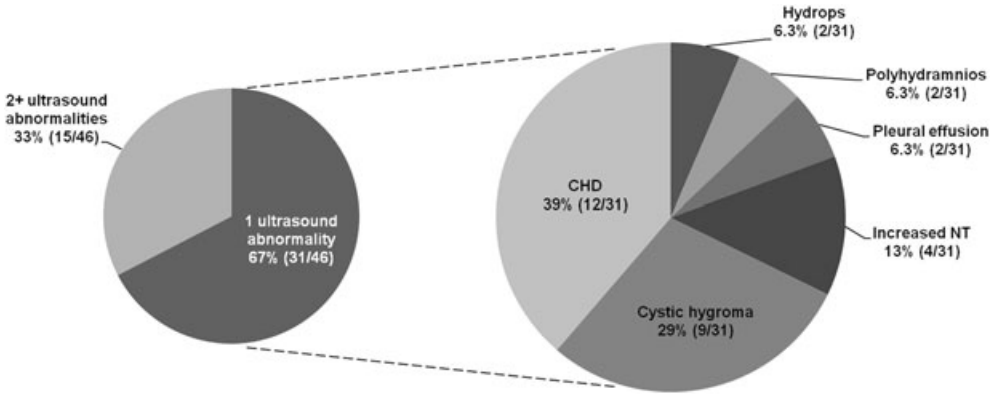

Figure 1 Distribution of ultrasound findings among molecularly confirmed NSD newborns. Two-thirds of NSD newborns confirmed positive by molecular testing had only one ultrasound abnormality. Congenital heart defect (CHD) was the most commonly detected ultrasound abnormality, followed by cystic hygroma and increased NT

abnormalities that could be difficult to identify by ultrasonography,[8] although actually may be because of the small cohort size in prior studies. Our findings suggest that *SOS1* is an important contributor to NSD-associated prenatal ultrasound findings and should be included in all prenatal testing for NSDs.

Limiting molecular testing to only fetuses with two or more abnormal features by ultrasound could have a large negative impact on the overall prenatal detection rate of NSDs, contrary to what previous studies have suggested.[8,9] Almost two-thirds of newborns confirmed by our laboratory to have a disease-causing variant had only one ultrasound abnormality, and there was no significant increase in detection rate in newborns with two ultrasound findings versus one ultrasound finding. The ultrasound findings of newborns with a NSD in our study were limited to physician-reported findings; thus, the percent of newborns with multiple anomalies may be underestimated here. However, as our study showed that 2/3 of molecularly positive newborns only had a single ultrasound finding, it is unlikely that unreported findings would dramatically alter these results.

While prenatal genetic testing for NSDs has utility regardless of the number of NSD-associated ultrasound findings, improvements can still be made in determining when these tests should be ordered. For example, a previous study suggested that prenatal diagnosis of NSDs may be improved by investigating fetal facial features using three-dimensional (3-D) ultrasonography,[9] a technique that is not commonly used in routine prenatal care. Larger cohorts with detailed pre- and postnatal clinical information, including this higher resolution ultrasonography, are needed to further refine the appropriate recommendations for prenatal testing of NSDs.

CONCLUSION

There is clinical utility in prenatal genetic testing for NSDs when prenatal ultrasonography identifies one or more NSD-associated abnormalities in euploid fetuses. This approach could improve the rate of NSDs prenatal detection, which would enhance family planning practices and postnatal management.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Certain ultrasonography abnormalities, such as cystic hygroma, increased nuchal translucency, and congenital heart defects, indicate an increased risk of a Noonan spectrum disorder (NSD) in a fetus.
- Current studies recommend prenatal testing for NSDs only when ultrasonography detected an increased nuchal translucency and at least one additional NSD-associated abnormality.

WHAT DOES THIS STUDY ADD?

- There is no significant difference in the detection rate of an NSD for fetuses with only one ultrasonography NSD-associated abnormality versus those with more.
- A testing strategy in which only fetuses with two or more NSD-associated abnormalities are tested will reduce the prenatal detection rate.

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SUPPORTING INFORMATION

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