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Prenatal cases with rare RIT1 variants causing severe fetal hydrops and death

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Abstract
We describe two clinical prenatal cases with rare de novo RIT1 variants, which showed more severe clinical manifestations than other Noonan Syndrome genotypes, resulting in fetal death. Extra attention is recommended when these variants are detected.

KEYWORDS
cystic hygroma, hydrops fetalis, Noonan syndrome, prenatal screening, RIT1

1 | INTRODUCTION

Here, we describe two clinical prenatal cases with rare *de novo* RIT1 variants, which showed more severe clinical manifestations than other Noonan Syndrome genotypes, resulting in fetal death. It is recommended that extra attention would be exercised when these variants are detected, and an appropriate patient counselling would be provided.

Noonan Syndrome (NS) is a multisystemic developmental disorder with an incidence of 1:1000–2500 in the general population.[1] It follows an autosomal dominant inheritance. 30–75% of patients with NS have an affected parent; nonetheless, NS can also be caused by a *de novo* variant. The disease has a wide spectrum of phenotypical features, which are associated with different genotypes. At present, 11 genes have been reported to cause NS when carrying a pathogenic variant.[2] Based on the chromosomal locations and genes involved, NS is divided into different types.[3]

The RIT1 (Ras-like without CAAX 1) gene is associated with Noonan Syndrome type 8 (NS8, OMIM #615355). The RIT1 gene is located on chromosome 1q22. The protein coded by RIT1 is involved in MAPK-dependent signaling pathways (involved in stress-mediated activation of p38-MK2-HSP27-AKT complex in cell-survival mechanism) and NGF (nerve growth factor) dependent nerve growth and differentiation.[4] Proteins coded by a mutated RIT1 gene cause hyperactivation of MAPK-ERK or transactivation of ELK pathways, participating in NS pathogenesis.[4] The gain-of-function mutations

of RIT1 are responsible for around 5% of all NS cases, making RIT1 one of the most common genetic causes of NS.[5]

The common distinctive characteristic in individuals with NS8 is hypertrophic cardiomyopathy, which is seen in at least 50% of the cases with a mutated RIT1 gene.[5-7] Congenital heart disease in NS gives one of the worst prognoses, as almost 25% of all NS patients die within a year due to heart failure.[1] Other more frequently observed characteristics among these patients are pulmonary stenosis and atrial or ventricular septal defects.[6,7]

Webbed and short neck is more common in RIT1 patients; however, short stature, pectus deformity, and intellectual disability are less common, compared with patients carrying mutations in other NS-associated genes.[4,8,9]

Prenatally, NS is suspected in cases with increased nuchal translucency (NT), a cystic hygroma, or hydrops fetalis.[10] Prenatal lymphatic malfunctions are more often observed in RIT1 NS cases,[6] which result in high birth weight and possible complications.

Here, we describe two prenatally detected NS cases with pathogenic RIT1 variants, which resulted in severe fetal hydrops and death.

2 | PRESENTATION OF CASE 1

A healthy nulliparous woman was referred to our tertiary fetal medicine clinic at the gestational age of (GA) 15 weeks +3 days. The family history revealed no congenital abnormalities and no consanguinity. The combined first-trimester screening (cFTR) was done in the primary care center at GA 12 weeks +5 days. An ultrasound scan showed a cystic hygroma with NT of 14.2 mm. The risk of trisomy 21 was 1:8. Chorionic villus sampling (CVS) was done on the following day. A normal male karyotype was reported in the chromosomal microarray. An early ultrasound of the fetal anatomy was performed at 15 weeks +3 days that showed a persistent cystic hygroma with NT of 14 mm and large jugular lymphatic sacs at each side of the neck measuring 24 × 16 mm for the left side and 20 × 10 mm for the right side. The fetus was not hydropic.

At GA 19 weeks +5 days, ultrasound scan showed smaller NT (12.4 mm) and jugular lymphatic sacs at both sides of neck, in the axial view of 7 × 10 mm on the left side and 5 × 8 mm on the right side. No other malformations were observed. The couple was offered a trio exome analysis and screened for variants in known Noonan-associated genes. The result showed a *de novo* heterozygous pathogenic RIT1 variant (c.268A>G (p. Met90Val), NM_006912.6). The couple was counseled about NS and their options. They decided to continue the pregnancy. At GA 28 weeks +6 days, an ultrasound showed normal growth with an estimated fetal weight of 1265 g. The enlarged NT and jugular lymphatic

sacs had disappeared. A new ultrasound was planned for GA 34 weeks, but the patient came in at GA 31 week +2 days due to reduced fetal movements. Bilateral severe hydrothorax (chylothorax) was revealed by the ultrasound. The fetal echocardiography was normal. Betamethasone was given for lung maturation. The intrauterine drainage of the hydrothorax was only possible to insert at one side. It confirmed chylothorax. However, the fetus died *in utero* the day after the drainage was performed. It had hypertelorism and a broad webbed neck, but otherwise no characteristic Noonan features. The couple did not opt for autopsy. A postnatal picture and ultrasound scan findings can be seen in Figure 1 as Case 1.

3 | PRESENTATION OF CASE 2

The patient was a nulliparous woman with a history of 2 years of infertility, but otherwise healthy, who became spontaneously pregnant. There were no congenital abnormalities and no consanguinity registered in the family history. The combined first-trimester screening was performed at GA 12 weeks +3 days, which showed a cystic hygroma with a large NT of 10.2 mm and mild cutaneous edema at the thorax and abdomen. The risk of trisomy 21 was 1:3. She was informed about the risks of genetic diseases, fetal malformations, and intrauterine fetal demise. She was offered a CVS analysis. A normal chromosomal microarray result was received. Due to the residual risk of genetic disease, she was offered trio exome analysis. An ultrasound of the fetal anatomy at GA 15 weeks +6 days still showed a cystic hygroma with NT of 15 mm. Furthermore, the fetus had pyelectasis of a slightly hyperechogenic left kidney.

At GA 18 weeks +2 days, a likely pathogenic, heterozygous *de novo* variant in the RIT1 gene (c.245T>G (p. Phe82Cys), NM_006912.6) was detected by trio exome analysis. The couple was counseled about NS. Ultrasound showed an NT of 18 mm, bilateral pyelectasis, and mild ascites. The patient decided to continue the pregnancy and was evaluated with ultrasound every 1–2 weeks due to increasing fetal hydrops. At GA 19 weeks +6 days, there was bilateral pyelectasis (hydronephrosis). Fetal echocardiography was normal.

At GA 23 weeks +6 days, she developed mild polyhydramnios with an amnion fluid index (AFI) of 25 cm. At GA 26 weeks +6 days, she still had mild polyhydramnios, but at GA 28 weeks +2 days, the polyhydramnios became severe, AFI 48 cm, and it started to bother the patient. At the same time, fetal hydrops kept increasing with moderate-to-severe hydrothorax, and ascites that enlarged the abdominal circumference to +12 in Z-score, giving an estimated fetal weight of 3000 g (equivalent to +130% of normal for the GA). The NT was 23 mm, and the fetus had large jugular lymphatic sacs on each side of the neck. The flow in the umbilical cord had an absent-end diastolic flow. The patient was offered therapeutic
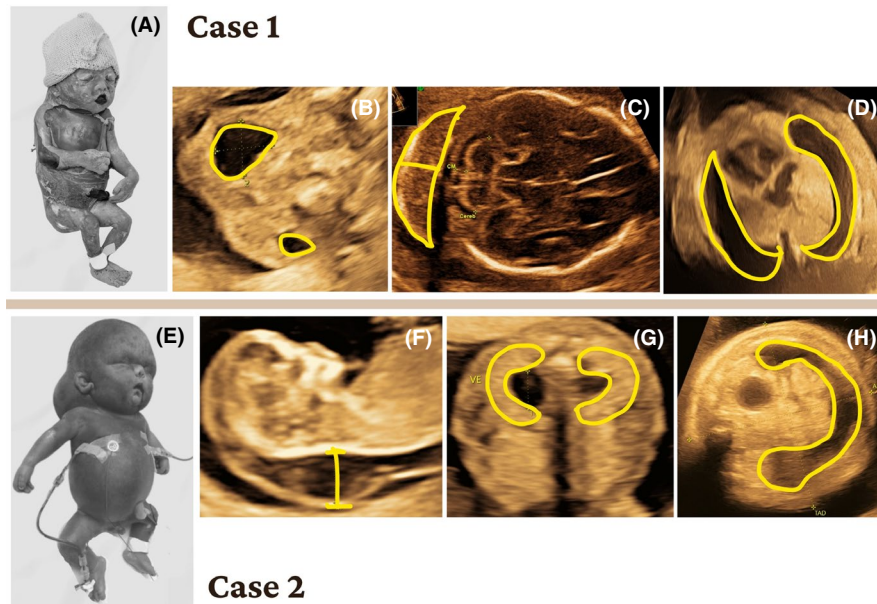
FIGURE 1 Postnatal pictures and ultrasound scans during pregnancy for Cases 1 and 2. Case 1: A, Postnatal picture of the baby, who died in utero at the gestational age of (GA) 31 week +5 days. It shows hypertelorism and a webbed neck. B, Jugular lymphatic sacs at GA 18 weeks, decreasing in size compared to an earlier scan. C, Large nuchal translucency (NT) / hygroma at GA 19 weeks +3 days, decreased from earlier scan. D, Bilateral pleural exudate at GA 31 week +2 days before insertion of drains. Case 2: E, Postnatal picture of the baby at GA 28 weeks +6 days showing universal hydrops with a large hygroma. F, Large NT / hygroma at GA 12 weeks +3 days. G, Kidneys with bilateral pyelectasis at GA 18 weeks +2 days. H, Enlarged abdominal circumference with ascites at GA 28 weeks +2 days

amniocentesis after 48 hours of fetal lung maturation with betamethasone.

The patient came back at GA 28 weeks +6 days for the amniocentesis and pediatric counseling. She reported that her condition had worsened during the previous 24 h with sleep problems and dyspnea. Further testing showed mirror syndrome resembling HELLP syndrome/preeclampsia. The amniocentesis was cancelled, and she had a subacute cesarean section. She was offered *ex utero intrapartum* treatment (EXIT) with intubation of the baby during cesarean before clamping of the umbilical cord. She declined that option, but otherwise, full treatment was planned. The newborn was severely hydropic at birth. It was intubated, ventilated, and given Curosurf® (poractant alfa). Bilateral pleural drainage was inserted. The treatment had no effect, and resuscitation was terminated after 27 min; the newborn died shortly after. It was severely hydropic with flattened facies. The birth weight of 2935 g was equivalent to +109% of normal for the GA and calculated to be >10 SD, based on Hadlock.[11] The couple did not opt for autopsy of the newborn. A postnatal picture and ultrasound scan findings can be seen in Figure 1 as Case 2.

4 | DISCUSSION

The two clinical prenatal cases described in this paper present rare RIT1 variants with similar, and different-than-usual NS manifestations. Both variants are situated at the

beginning of exon 5 of the RIT1 gene. The majority of known NS-associated pathogenic variants cluster in exons 4 and 5 (ClinVar).[6] These variants (in switch II region of RIT1), described by Milosavljevic et al.[6] lead to a change in amino acid which is in a conserved sequence of the gene.

The RIT1 variant c.268A>G (p. Met90Val) identified in Case 1, was previously detected in 4 prenatal cases, which all presented with hydrops fetalis (Table 1). In Case 2, a RIT1 variant c.245T>G (p. Phe82Cys) was detected that was previously described once in a fetus with hydrops fetalis, CNS malformations, and congenital heart defect associated with NS.[10]

A number of publications describe postnatally identified variants in the same codons as the two RIT1 variants in Cases 1 and 2; however, these involved different nucleotides and resulted in a different amino acid after substitution, which presented milder Noonan characteristic symptoms.[8,9,12-14] To the best of our knowledge, there are no exact postnatally identified variants described in the literature matching the two cases we present. For all the cases with prenatally identified variants in RIT1, matching the variants described in Cases 1 and 2, intrauterine fetal demise or death during labor was reported at GA 18–32 weeks (Table 1). This suggests more severe clinical manifestations than other Noonan genotypes and a worse prognosis in cases with the RIT1 variants we have described in this case report.

A recently published study, learning the genetic causes of non-immune hydrops fetalis, showed that 29% of the cases were solely due to gene variants in MAPK-dependent

TABLE 1 Prenatal and postnatal fetal as well as maternal phenotypic characteristics and clinical outcome for two cases described here, and other published cases with corresponding identical variants in RIT1 gene

Characteristic/Case	Case 1	Stevens et al. 2017	Milosavljevic et al. 2016	Matyášová et al. 2019	Quinlan-Jones et al. 2019	Case 2	Yates et al. 2017
Genomic RIT1 variant (NM_006912.5)	c.268A>G					c.245T>G	
Protein variant (NP_008843.1)	p. Met90Val					p. Phe82Cys	
De novo	+	+	+	+	+	+	+
Fetal (prenatal)							
Increased nuchal translucency	+	[d]	+	+		+	
Cystic hygroma	+	+	+		+	+	+
Large jugular lymphatic sacs	+	+	+			+	
Hydrops fetalis	+	+	+[e]	+	+	+	+
Bilateral hydrothorax/ pleural effusion	+	+	+			+	
Ascites	No		+	+	+	+	
Skin edema	No				+	+	
Severe generalized edema/ anasarca	No	+	+			+	
Hydronephrosis/pyelectasis	No	+	[a]			+[b]	
Flattened facies	No	+				+	
Cardiac defect	No	No	No			No	+
CNS malformations	No	No	No			No	+[c]
Maternal							
Polyhydramnios	No	+	+			+	
Amnioreduction Procedure	No	Several	Once			Planned	
HELLP syndrome/preeclampsia/ Mirror syndrome	No	No	+			+	
Fetal death (GA)	31+5 IUFD	30 IUFD	26+4 during labor	IUFD	IUFD	28+6 after CS	18
Fetal/child (postnatal)							
High (birth) weight	No		>2.5 SD			>10 SD	
Hypertelorism	+		+				
Low set ears	No		+			No	
Broad short webbed neck	+		+			+	
Other					p	[a]	
Pathological examination	NP		NP		+	NP	

+—the characteristic was present, no—the characteristic was not present, an empty square—no information, GA—gestational age in weeks and days (eg, 31+5 means 31 week +5 days), CS—caesarian section, NP—not permitted/not performed.

If GA or other information about the fetal demise is not written, it is unknown or not provided by the authors.

[a]—Duplication of renal collection system.

[b]—Hyperechogenic left kidney.

[c]—Dandy-Walker malformation.

[d]—cFTR not performed, 1st scan at GA 21.

[e]—Severe at GA 25, p—superior vena cava, atrial septal defect, bilateral talipes.

signaling pathways.[15] All of them were *de novo* variants, including a RIT1 variant. It indicates that Noonan-associated gene variants are a rather frequent finding in cases with hydrops fetalis. Furthermore, that study supports the use of exome sequencing for prenatal diagnosis in cases with hydrops fetalis as the ones described here.

Based on our described clinical cases and the outcomes of other recent studies, it is advised to remain attentive about the prognosis in cases where a cystic hygroma or hydrops fetalis and a rare *de novo* RIT1 variant are identified prenatally, as it might result in a more-severe-than-usual Noonan outcome resulting in fetal death. Thus, it is recommended that the patients be counselled about these potential risks.

5 | ETHIC STATEMENT

Verbal and written informed consent was obtained from the patients for publications of their cases.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

Ieva Miceikaite: contributed to conception and design for this manuscript, collection of the data, wrote and edited the paper, prepared the table and the figure, and approved the final version to be published. Geske Sidsel Bak: contributed to conception and design, acquisition of data, including ultrasound pictures, and drafting the case descriptions. Revised the paper and approved the final version to be published. Martin Jakob Larsen: contributed to conception and design, performed the analysis and interpretation of the data, critically revised the draft, and approved the final version to be published. Britta Schlott Kristiansen: contributed to acquisition of the data, critically revised the draft, and approved the final version to be published. Pernille Mathiesen Torring: contributed to conception and design, and acquisition of data. Revised the paper and approved the final version to be published.

DATA AVAILABILITY STATEMENT

The authors confirm that the main data supporting the findings of this study are available within the article. The other data are not publicly available to protect the privacy of the patients.

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