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 gene 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.6-7 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 the 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
amniocentesis after 48 hours of fetal lung maturation with 

treatment.

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 hydptic at birth. It was intubated, ventilated, and 
given Curosurf® (poractan 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 hydptic 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. 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). These variants (in switch II region of RIT1), de- 
scribed by Milosavljevic et al. 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 previ- 
ously described once in a fetus with hydrops fetalis, CNS mal-
formations, and congenital heart defect associated with NS.

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 re- 
sulted in a different amino acid after substitution, which pre-
sented milder Noonan characteristic symptoms. To the 
best of our knowledge, there are no exact postnatally iden-
tified 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
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.

### 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             | +               | +                   | +                         | +                      | +                         | +               | +                |
| Cystic hygroma                            | +               | +                   | +                         | +                      | +                         | +               | +                |
| Large jugular lymphatic sacs              | +               | +                   | +                         | +                      | +                         | +               | +                |
| Hydrops fetalis                           | +               | +                   | +                         | +                      | +                         | +               | +                |
| Bilateral hydrothorax/pleural effusion    | +               | +                   | +                         | +                      | +                         | +               | +                |
| Ascites                                   | No              | +                   | +                         | +                      | +                         | +               | +                |
| Skin edema                                | No              |                     |                           | +                      | +                         | +               | +                |
| Severe generalized edema/anasarca         | No              | +                   | +                         | +                      | +                         | +               | +                |
| Hydromecephrosis/pyelectasis              | No              | +                   | +                         | +                      | +                         | +               | +                |
| Flattened facies                          | No              | +                   | +                         | +                      | +                         | +               | +                |
| Cardiac defect                            | No              | No                  | No                        | No                     | No                        | +               |                   |
| CNS malformations                         | No              | No                  | No                        | No                     | No                        | No              | +                |
| 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           | 18               |
| Fetal/child (postnatal)                   |                 |                     |                           |                        |                           |                 |                  |
| High (birth) weight                       | No              | +                   | +                         | >2.5 SD                | >10 SD                    | +               | +                |
| Hypertelorism                              | +               | +                   | +                         | +                      | +                         | +               | +                |
| Low set ears                              | No              | +                   | +                         | +                      | +                         | +               | +                |
| Broad short webbed neck                   | +               | +                   | +                         | +                      | +                         | +               | +                |
| Other                                     |                 |                     |                           |                        |                           | p               |                   |
| Pathological examination                  | NP              | 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.

*—Duplication of renal collection system.

**—Hyerechogenic left kidney.

***—Dandy-Walker malformation.

****—cFTR not performed, 1st scan at GA 21.

*****—Severe at GA 25, p—superior vena cava, atrial septal defect, bilateral talipes.
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 \textit{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.

ACKNOWLEDGEMENTS

Authors want to express their special thanks to the patients who allowed the publication of these clinical cases. This paper includes data from the PhD research project, which was supported by the grants from the following funds: Aase og Ejnar Danielsens Fond (grant no. 19-10-0259) and A.P. Møller—Fonden til Lægevidenskabens Fremme (grant no. 19-L-0281). Published with written consent of the patient.

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.

ORCID

Ieva Miceikaite $https://orcid.org/0000-0002-5188-7647$

Pernille Mathiesen Torring $https://orcid.org/0000-0002-7303-7619$

REFERENCES

1. Roberts AE, Allanson JE, Tartaglia M, Gelb BD. Noonan syndrome. \textit{Lancet}. 2013;381(9863):333-342.
2. Cai J, Li H. A novel RIT1 mutation causes deterioration of Noonan syndrome-associated cardiac hypertrophy. \textit{EBioMedicine}. 2019;42:6-7.
3. Allanson JE, Roberts AE. Syndrome N. 2001 Nov 15 [Updated 2019 Aug 8]. In: GeneReviews® [Internet]; 1993-2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1124/
4. Cavè H, Caye A, Ghedira N, et al. Mutations in RIT1 cause Noonan syndrome with possible juvenile myelomonocytic leukemia but are not involved in acute lymphoblastic leukemia. \textit{Eur J Hum Genet}. 2016;24(8):1124-1131.
5. Aoki Y, Niinohri T, Inoue S, Matsubara Y. Recent advances in RASopathies. \textit{J Hum Genet}. 2016;61(1):33-39.
6. Milosavljevic D, Overwater E, Tammenga S, et al. Two cases of RIT1 associated Noonan syndrome: further delineation of the clinical phenotype and review of the literature. \textit{Am J Med Genet A}. 2016;170(7):1874-1880.
7. Calcagni G, Baby A, Lepri FR, Marino B, Tartaglia M, Diggio MC. Congenital heart defects in Noonan syndrome and RIT1 mutation. \textit{Genet Med}. 2016;18(12):1320.
8. Yaito M, Niinohri T, Mizuno S, et al. Spectrum of mutations and genotype-phenotype analysis in Noonan syndrome patients with RIT1 mutations. \textit{Hum Genet}. 2016;135(2):209-222.
9. Bertola DR, Yamamoto GL, Almeida TF, et al. Further evidence of the importance of RIT1 in Noonan syndrome. \textit{Am J Med Genet A}. 2014;164A(11):2952-2957.
10. Yates CL, Monaghan KG, Copenheaver D, et al. Whole-exome sequencing on deceased fetuses with ultrasound anomalies: expanding our knowledge of genetic disease during fetal development. \textit{Genet Med}. 2017;19(10):1171-1178.
11. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements–a prospective study. \textit{Am J Obstet Gynecol}. 1985;151(3):333-337.
12. Aoki Y, Niinohri T, Banjo T, et al. Gain-of-function mutations in RIT1 cause Noonan syndrome, a RAS/ MAPK pathway syndrome. \textit{Am J Hum Genet}. 2013;93(1):173-180.
13. Gos M, Fahiminiya S, Poznański J, et al. Contribution of RIT1 mutations to the pathogenesis of Noonan syndrome: four new cases and further evidence of heterogeneity. \textit{Am J Med Genet A}. 2014;164A(9):2310-2316.
14. Kouz K, Lissewski C, Spranger S, et al. Genotype and phenotype in patients with Noonan syndrome and a RIT1 mutation. \textit{Genet Med}. 2016;18(12):1226-1234.
15. Sparks TN, Lianoglou BR, Adami RR, et al. Exome sequencing for prenatal diagnosis in nonimmune hydrops fetalis. \textit{N Engl J Med}. 2020;383(18):1746-1756.

How to cite this article: Miceikaite I, Bak GS, Larsen MJ, Kristiansen BS, Torring PM. Prenatal cases with rare RIT1 variants causing severe fetal hydrops and death. \textit{Clin Case Rep}. 2021;9:e04507. $https://doi.org/10.1002/ccr3.4507$