# Comparative Assessment of Prenatal and Postnatal Molecular Testing for Noonan Syndrome and Related Conditions

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## I. Introduction

- Noonan syndrome belongs to a group of closely related conditions, including Noonan syndrome with multiple lentigines, Noonan syndrome-like disorder with loose anagen hair, Costello syndrome, and cardiofaciocutaneous syndrome.
- Noonan syndrome and related disorders (NSRC) represent one of the most common syndromic causes for congenital heart defects.[1] In addition, they share features of short stature, distinct facial appearance, congenital heart defects, cryptorchidism, skin pigmentation anomalies, variable degrees of intellectual impairment, and risk of malignancies.[2] Frequent prenatal anomalies associated with NSRC involve lymphatic dysplasia, most commonly increased nuchal translucency or cystic hygroma, polyhydramnios, and congenital heart defects.[3]
- Due to similarities in clinical presentation with other conditions, as well as marked variable expressivity, the diagnosis of NSRC can be challenging. Molecular genetic testing is an essential tool in diagnostic process of NSRC patients and in the assessment of fetuses with associated ultrasound anomalies.
- NSRC have an autosomal dominant pattern of inheritance and are typically caused by gain-of-function mutations enhancing RAS/MAPK signaling.[4]
- Mutations in *BRAF, HRAS, KRAS, MAP2K1, MAP2K2, PTPN11, RAF1, SOS1,* and *SHOC2* genes have a well-established role in NSRC etiology, and are included in Integrated Genetics GeneSeq®: Cardio Noonan Syndrome and Related Conditions Profile and Prenatal Noonan Syndrome panels.
- This study is a comparative assessment of NSRC prenatal and postnatal molecular testing outcomes using Prenatal Noonan Syndrome and GeneSeq®: Cardio Noonan Syndrome and Related Conditions Profile panels.
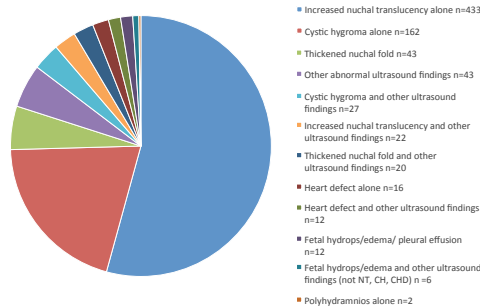
## II. Methods

- Data collected from 1,202 cases comprising 845 prenatal and 357 postnatal specimens were analyzed retrospectively.
- Sequence assessment was performed using the Agilent Sure Select enrichment method and the Illumina next-generation sequencing (NGS) platform. Regions of analysis included all exons and splice junctions of *BRAF, HRAS, KRAS, MAP2K1, MAP2K2, PTPN11, RAF1,* and *SOS1* genes, and exon 2 of the *SHOC2* gene.
- Sanger sequencing was used to confirm variants identified by NGS.
- Absence of significant maternal cell contamination was ensured for all prenatal cases.
- For prenatal specimens, karyotype and microarray results were assessed when available.
- Clinical significance of identified variants was interpreted using internally developed and validated scoring algorithms. An internal 7-point scale subdivides variants of uncertain significance (VUS) into three subgroups: possibly pathogenic variant, variant of uncertain significance and possibly benign variant (**Figure 1**).
- Identification of pathogenic and likely pathogenic variants was considered a positive result.

### Figure 1. Variant Classification Scale

| ACMG variant classification | Pathogenic variant | Likely pathogenic variant | Variant of uncertain significance (VUS) | | | Likely benign variant | Benign variant |
|---|---|---|---|---|---|---|---|
| Integrated Genetics variant classification | Pathogenic variant | Likely pathogenic variant | Possibly pathogenic variant | Variant of uncertain significance | Possibly benign variant | Likely benign variant | Benign variant |

### Figure 2. Clinical indications for prenatal NSRC Studies



- Increased nuchal translucency alone n=433
- Cystic hygroma alone n=162
- Thickened nuchal fold n=43
- Other abnormal ultrasound findings n=43
- Cystic hygroma and other ultrasound findings n=27
- Increased nuchal translucency and other ultrasound findings n=22
- Thickened nuchal fold and other ultrasound findings n=20
- Heart defect alone n=16
- Heart defect and other ultrasound findings n=12
- Fetal hydrops/edema/ pleural effusion n=12
- Fetal hydrops/edema and other ultrasound findings (not NT, CH, CHD) n =6
- Polyhydramnios alone n=2

## III. Results

**Prenatal specimens:**

- The most commonly reported clinical indications were increased nuchal translucency (51.2%) and cystic hygroma (19.2%) (**Figure 2**).
- Chromosomal studies (karyotyping and/or array) were done in 84.3% (712/845) of cases and normal results were obtained in over 97% (694/712).
- The total diagnostic yield was 3.6 % (30/845). However, the diagnostic yield varied when prenatal cases are stratified by the clinical indication. For the most common clinical indications, the diagnostic yield becomes 1.4% (6/433) for increased nuchal translucency and rises to 11.7% (19/162) for cystic hygroma.
- The gene-specific distribution shows that 80% (24/30) of pathogenic and likely pathogenic variants were found in *PTPN11* (**Figure 3**). Additionally, pathogenic and likely pathogenic variants were detected in *RAF1* (3), *SOS1* (1), *BRAF1* (1) and *HRAS* (1), but at a significantly lower rate.
- A VUS rate of 5.7% was observed, with the highest rate of 39.6% (19/48) in the *SOS1* gene.

**Postnatal specimens:**

- The diagnostic yield was 21.3% (76/357).
- The gene-specific distribution for postnatal specimens showed that *PTPN11* is the highest contributor to the diagnostic yield by harboring 73.3% (55/75) of pathogenic and likely pathogenic variants. In all genes but *MAP2K2*, pathogenic or likely pathogenic variants were identified: *RAF1* (5), *SOS1* (5), *BRAF1* (3), *KRAS* (2), *MAP2K1* (2), *SHOC2* (2), and *HRAS* (1) (**Figure 4**).
- A VUS rate of 12.6% was observed, with the highest rate of 24.4% (11/45) in the *SOS1* gene.
- Positive rates were compared among patients referred by different medical institutions and a wide range was observed, from 0% to 47% (**Table 1**).

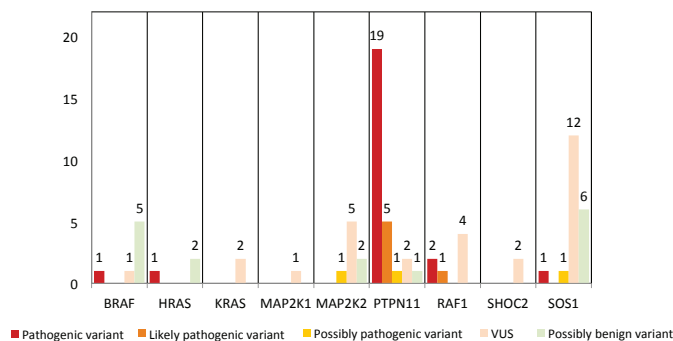### Figure 3. Gene-specific variant distribution in prenatal specimens



Pathogenic variant ■ Likely pathogenic variant ■ Possibly pathogenic variant ■ VUS ■ Possibly benign variant

### Figure 4. Gene-specific variant distribution in postnatal specimens



Pathogenic variant ■ Likely pathogenic variant ■ Possibly pathogenic variant ■ VUS ■ Possibly benign variant
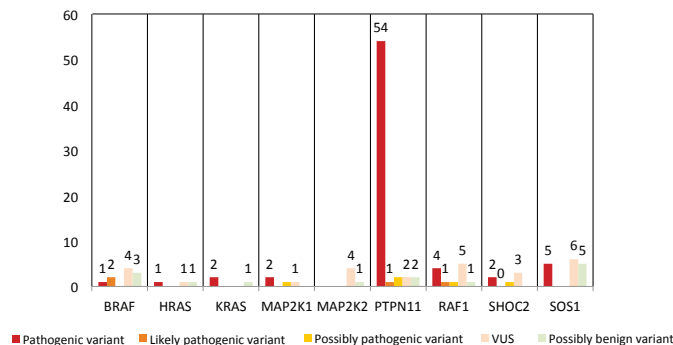
### Table 1. Rate of positive outcomes observed in patients referred by different medical institutions

| Institution | Cases with positive results | Total number of cases | Positive rate |
|---|---|---|---|
| A | 30 | 139 | 21.58% |
| B | 9 | 19 | 47.40% |
| C | 3 | 22 | 13.60% |
| D | 2 | 13 | 15.38% |
| E | 1 | 5 | 20.00% |
| F | 0 | 8 | 0.00% |
| G | 1 | 3 | 33.33% |
| H | 13 | 40 | 32.50% |
| I | 2 | 3 | 66.66% |
| J | 6 | 35 | 17.14% |
| K | 10 | 70 | 14.29% |

## IV. Discussion

- Method of ascertainment has an important impact on diagnostic yield. The diagnostic yield for postnatal samples, i.e. in patients exhibiting clinical features of NSRC, is expected to be much higher in comparison with prenatal samples, where the indication for testing is less specific. Our comparative assessment demonstrates a close to 6 fold difference in positive rate between postnatal (21.3%) and prenatal (3.6%) cases (**Figure 5**). It is important to note, however, that diagnostic yield in prenatal specimens varies depending on the ultrasound findings. A positive rate of 11.7% was observed for the prenatal cases with cystic hygroma.
- The gene-specific distribution of pathogenic and likely pathogenic mutations was similar between post- and prenatal groups, with *PTPN11* being the highest contributor (postnatal 73.3%, prenatal 80%), followed by *RAF1* (6.6%) and *SOS1* (6.6%) in postnatal, and *RAF1* (10%) in prenatal cases. No pathogenic and likely pathogenic variants were identified in *MAP2K2* genes in both groups, or in *MAP2K1, KRAS,* and *SHOC2* in the prenatal group.
- A two-fold difference in VUS rate was observed between postnatal (12.6%) and prenatal (5.7%) cases. Interestingly, *SOS1* harbored most of the VUSs in both groups (postnatal 24.4%, prenatal 39.6%).
- When patients were stratified by the referring clinic, a wide range of positive rate was observed. This data could be interpreted as a reflection of clinician-specific effect in patient ascertainment.
- Fifty cases were available to determine the origin of an identified variant. The results showed that 90.5% (19/21) of the pathogenic and likely pathogenic variants originated de novo, whereas most of the VUSs were inherited (79.3%) (**Table 2**). Clinical interpretation of inherited VUSs is frequently hindered by the lack of detailed clinical information/genetic assessment in a carrier parent.

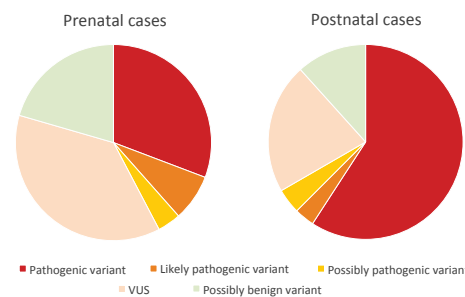### Figure 5. Variant-specific distribution in postnatal and prenatal specimens



Prenatal cases — Postnatal cases

■ Pathogenic variant ■ Likely pathogenic variant ■ Possibly pathogenic variant
■ VUS ■ Possibly benign variant

### Table 2. Parental follow up testing

| Variant classification | Variant origin | | | |
|---|---|---|---|---|
| | de novo | MAT | PAT | |
| Pathogenic | 17 | 2 | | |
| Likely pathogenic | 2 | | | |
| Possibly pathogenic | 4 | | 1 | |
| Uncertain significance | 2 | 9 | 8 | |
| Possibly benign | | 3 | 2 | |
| | 25 | 14 | 11 | Total 50 |

## V. Conclusion

Our data shows that variants detected in postnatal and prenatal samples have similarities in gene-specific variant distribution trends. Ascertainment, including prenatal or postnatal context, clinical indication and assessment, is an important factor in defining the diagnostic yield.

## VI. References

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4. Tidyman and Rauen. The RASopathies: Developmental Syndromes of Ras/MAPK Pathway Dysregulation. *Curr Opin in Genet & Develop* 19.3 (2009):230-36.

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