I. Introduction

- Noonan syndrome belongs to a group of closely related conditions, including Noonan syndrome with multiple lentigines, Noonan-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. 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.
- Frequent prenatal anomalies associated with NSRC involve lymphatic dysplasia, most commonly increased nuchal translucency or cystic hygroma, polyhydramnios, and congenital heart defects.
- 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.
- 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 GeneSet® 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 GeneSet® 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 SureSelect 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, SOS1, and SOST genes, and exons 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.

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 12.7% (19/156) 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), RAF1 (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), BRAF (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).

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 postnatal 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 were de novo, whereas most of the VUSs were inherited (76.3%) (Table 2). Clinical interpretation of inherited VUSs is frequently hindered by the lack of detailed clinical information/genetic assessment in a carrier parent.

Figure 3. Gene-specific variant distribution in prenatal specimens

Figure 4. Gene-specific variant distribution in postnatal specimens

Table 1. Rate of positive outcomes observed in patients referred by different medical institutions

Table 2. Parental follow up testing

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