Clinical Case Reports

CASE REPORT

Nonspecific phenotype of Noonan syndrome diagnosed by whole exome sequencing

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Key Clinical Message

Noonan syndrome is a genetically heterogeneous condition primarily due to missense mutations in *PTPN11*. Prenatal diagnosis is typically made in a fetus with increased nuchal translucency and normal karyotype. We demonstrate the ability of whole exome sequencing to make prenatal diagnoses that would not have been made from phenotype alone.

Introduction

Noonan syndrome (NS) is a genetically heterogeneous condition characterized by short stature, distinctive facies, congenital heart defects, hypertrophic cardiomyopathy, and learning disabilities. It has an estimated prevalence of 1:1000–2500 births. NS exhibits autosomal dominant inheritance, and approximately half of the cases arise due to de novo mutations [1].

Missense mutations in *PTPN11* account for approximately 50% of NS cases and are activating mutations in the nonreceptor-type protein tyrosine phosphatase SHP-2 [2]. Mutations in *SOS1*, *RAF1*, *KRAS*, *MAP2K1*, *BRAF*, *NRAS*, and *RIT1* have also been identified in NS [3, 4]. Furthermore, mutations in other genes including *SHOC2* and *CBL* have been found in patients with NS-like syndromes [4]. All of these genes encode proteins that participate in signaling through the RAS-MAPK signal transduction pathway. Other genetic disorders resulting from dysregulation of this pathway include Leopard syndrome (*PTPN11*, *RAF1*), Costello syndrome (*HRAS*, *KRAS*, *BRAF*, and *MAP2K1*), and cardiofaciocutaneous syndrome (*KRAS*, *BRAF*, *MAP2K1*, and *MAP2K2*) [3, 4].

The diagnosis of NS is readily made postnatally, but increasingly prenatal diagnoses have been made because of the association of NS with an increased nuchal translucency (NT) or cystic hygroma, and the availability of prenatal clinical molecular genetic testing with panels of genes associated with NS.

We present a diagnosis of NS in a fetal demise using whole exome sequencing (WES) that demonstrates the possible nonspecific prenatal phenotype in NS.

Case Report

A 31-year-old, nonconsanguineous gravida 1, para 0 woman was referred for genetic consultation for ultrasound findings of polydactyly and pyelectasis.

Routine first trimester serum screening was normal. Nuchal translucency (NT) measured 2.0 mm, at the 75th percentile for gestational age. Routine prenatal ultrasound at 21 weeks gestation demonstrated unilateral post axial polydactyly on the right foot, and the left foot was difficult to visualize with the impression of syndactyly or a missing toe. There was mild bilateral pyelectasis at 4 mm. There was noted to be "subjectively generous" amniotic fluid with maximal vertical pocket measuring 7.4 cm. Because of these findings, an amniocentesis was performed that demonstrated a normal female 46, XX karyotype with a normal chromosome microarray.

Repeat ultrasound at 22 weeks 2 days gestation demonstrated no evidence of polydactyly. However, there was

syndactyly of the feet bilaterally, right pyelectasis of 5 mm with a normal appearing left kidney. There was no evidence of polyhydramnios. The remainder of fetal anatomy appeared normal.

At 32 weeks 2 days gestation, the patient presented to an outside hospital with decreased fetal movement for 2 days. Ultrasound revealed absent fetal cardiac activity. A postmortem examination showed right unilateral Simian crease, hypertelorism, micrognathia, and sacral dimple (Fig. 1). The extremities appeared normal with no evidence of polydactyly or syndactyly. Autopsy failed to identify any gross or microscopic congenital abnormalities and specifically no evidence of pulmonic stenosis, cardiomyopathy, or renal pathology. The cause of fetal demise was also not clear based on the postmortem examination.

Whole exome sequencing of the proband/parent trio was used to determine a definitive diagnosis. WES resulted in an average of ~12.1 Gb of sequence per sample. Mean coverage of captured regions was 88× per sample, with >97.4% covered with at least 10× coverage, an average of ~92% base call quality of Q30 or greater, and an overall average mean quality score of <Q35. Stepwise filtering removal of common SNPs, intergenic and 3′/5′ UTR variants, nonsplice-related intronic variants, and synonymous variants resulted in ~4400 variants in the proband. Family history inheritance model filtering based on autosomal and X-linked dominant and recessive inheritance models revealed 84 genes with 101 alterations (Table 1). Manual review of each alteration to rule out sequencing artifacts and polymorphisms along with medical interpretation to rule out genes lacking clinical overlap

Figure 1. Postmortem findings of hypertelorism and micrognathia.

with the patient's evaluated phenotype resulted in two genes with two unique alterations (Table 1). A de novo heterozygous N58K pathogenic variant in *PTPN11* associated with NS was identified and confirmed by fluorescence dideoxy sequencing of the proband and both parents. In addition, a single heterozygous missense change in the *WDR35* gene associated with autosomal recessive short rib-polydactyly syndrome and cranioectodermal dysplasia was identified. However, this was considered unlikely to be associated with the phenotype due to detection of only one potential mutation and the lack of correlation with the phenotype.

Discussion

The *PTPN11* N58K mutation identified in the proband has been previously associated with NS [5] along with two other missense mutations of this residue, N58D and N58H [6]. The mutation lies in the *PTPN11* N-SH2 domain, where the majority of the identified NS mutations cluster. A study of genotype–phenotype correlation found that NS patients with *PTPN11* mutations have a higher incidence of pulmonic stenosis and lower incidence of hypertrophic cardiomyopathy than NS patients without *PTPN11* mutations [7]. The same study did not find differences in frequency of short stature, pectus deformities, cryptorchidism, or developmental delay in NS patients with *PTPN11* compared to NS patients without *PTPN11* mutations. Finally, the subgroup of patients with mutations in the N-SH2 domain did not have distinctive clinical manifestations compared to NS patients with mutations in other *PTPN11* domains [5,7].

This case highlights the challenges of consistently making the prenatal diagnosis of NS. NS is increasingly diagnosed prenatally, but this is most often in cases with an increased nuchal translucency (NT) or cystic hygroma. In this case, there was no prenatal evidence of an increased NT or cardiac anomaly by either ultrasound or postmortem examination. This fetus has none of the cardinal features of NS to suggest the diagnosis, and until WES data were analyzed, the diagnosis was not suspected.

Table 1. Number of genes with novel variants identified from whole exome sequencing after filtering of results and manual review of the genes. The number of variants after filtering is listed in parentheses.

	Filtering results	Manual review	Resulting genes of interest
Homozygous	6 (6)	0 (0)	0 (0)
Compound heterozygous	12 (25)	0 (0)	0 (0)
Heterozygous	53 (57)	2 (2)	1 (1)
X-linked genes	13 (13)	0 (0)	0 (0)
Total genes	84 (101)	2 (2)	1 (1)

Increased NT, cystic hygroma, or increased nuchal fold raise the suspicion for NS in a fetus with a normal karyotype [8]. However, increased NT is not a sine qua non for NS. In a retrospective study of 47 patients with a molecular diagnosis of NS, 22 of those patients had triple screen and measurement of NT [9]. Of those, only 9/22 (41%) had increased NT. Other identified prenatal findings included polyhydramnios in 18/47 (38%), hydrothorax and/or pleural effusion in 5/47 (11%), heart defect in 4/47 (9%), and pyelectasis in 2/47 (4%). However, retrospectively, the majority of patients had no abnormal ultrasound findings identified.

Physical examination of the fetus revealed facial features of hypertelorism and micrognathia that can be associated with NS, but these are clearly nonspecific [1]. A study of 32 subjects with NS and *PTPN11* mutations analyzing the craniofacial dysmorphisms, demonstrated low set ears, low posterior hair line, down-slanting palpebral fissures, ptosis, inverted hair, abnormal auricles, epicanthus, hypertelorism, short neck, and pterygium colli as variable facial features that can be associated with NS [5]. To the best of our knowledge, the simian crease and sacral dimple we observed in this case are not frequently observed with NS.

WES in this case revealed a diagnosis that would otherwise not have been suspected based on the nonspecific clinical presentation prenatally. It is likely that with the increasingly utilization of WES we will continue to expand the prenatal phenotype of genetic syndromes as they are currently defined. This will pose significant challenges in the prenatal setting as we identify variants of uncertain significance in prenatal cases that do not match the classical phenotypic presentations of these conditions. We will likely struggle with interpretation of these results and should therefore consider first analyzing fetal demises or banked prenatal samples from pregnancies that are no longer ongoing and for which additional outcome data may be available before proceeding with studies to utilize WES in the clinical prenatal setting.

Acknowledgment

Conflicts of Interest

Eden Haverfield is an employee of GeneDx and Wendy K. Chung is a consultant to BioReference Laboratories.

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