Prenatal overgrowth and polydramnios: Would you think about Noonan syndrome?

Benedetta Beltrami1 | Jacopo Cerasani2 | Alessandra Consales2 | Roberta Villa1 | Nicoletta Resta3 | Daria Carmela Loconte3 | Simona Boito4 | Luca Caschera5 | Laura Bassi2 | Lorenzo Colombo2 | Maria Iascone6 | Maria Francesca Bedeschi1

1Medical Genetics Unit, Fondazione IRCCS Ca’Granda Ospedale Maggiore Policlinico, Milan, Italy
2Neonatal Intensive Care Unit (NICU), Department of Clinical Science and Community Health, Università degli Studi di Milano and Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy
3Department of Biomedical Sciences and Human Oncology (DIMO), Medical Genetics, University of Bari “Aldo Moro”, Bari, Italy
4Fetal Medicine and Surgery Service, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy
5Neuroradiology Unit, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy
6Laboratory of Medical Genetics, Ospedale Papa Giovanni XXIII, Bergamo, Italy

Correspondence
Maria Francesca Bedeschi, Medical Genetics Unit, Fondazione IRCCS Ca’Granda Ospedale Maggiore Policlinico, Via della Commenda 12, Milano, Italy.
Email: mariafrancesca.bedeschi@policlinico.mi.it

Funding information
European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability, Grant/Award Number: 3HP-HP-FPA ERN-01-2016/739516

Abstract
We report on a child with prenatal findings of increased nuchal translucency, polydramnios, ascites, and overgrowth. At birth, she presented length >97th centile, minor facial anomalies, megalencephaly, and Wolff–Parkinson–White syndrome. Whole-exome sequencing showed a pathogenic variant in the NRAS gene, but no mutations were found in PI3K/AKT/mTOR pathway genes.

Keywords
cardiovascular disorders, genetics, neurology, obstetrics and gynecology, pediatrics and adolescent medicine

1 | INTRODUCTION

Noonan syndrome (NS, MIM # 163950) is a common genetic disorder. It is characterized by the presence of short stature, minor facial anomalies, congenital heart defects, variable degrees of developmental delay and intellectual disability, skeletal malformations, and coagulation defects.1,2

The incidence of NS is estimated to be between 1:1000 and 1:2500 live births; there is no difference between sexes.3

NS is caused by germline mutations in genes involved in the Ras-mitogen-activated protein kinase (RAS-MAPK) pathway, while somatic mutations are frequently found in various types of tumors. This pathway is implicated in cell proliferation, differentiation, and survival.

PTPN11 (MIM * 176876) was the first gene to be associated with NS, and it is mutated in about 50% of individuals affected by Noonan syndrome.

Other genes that can be involved are SOS1 (MIM * 182530), RAF1 (MIM * 164760), RIT1 (MIM * 609591), KRAS (MIM * 190070), NRAS (MIM * 164790), BRAF (MIM * 64757) MAP2K1 (MIM * 176872), and LZTR1 (MIM * 600574).
Noonan syndrome has an autosomal dominant model of inheritance, with the exception of certain mutations of *LZTR1* gene.\(^3,4\)

Congenital heart defects are a major problem in NS. The most frequent anomaly is pulmonary valve stenosis, whereas hypertrophic cardiomyopathy, atrial or ventricular septal defects, tetralogy of Fallot, and branch pulmonary artery stenosis are described in a minority of cases.\(^5\)

Megalencephaly is defined as a head circumference 2 Standard Deviations (SD) above the age- and sex-related mean, caused by an increased growth of cerebral structures. This is due to disruptions of the normal brain development, especially during neuronal proliferation and migration. Megalencephaly can be evident at birth, or it can develop over time, during infancy or childhood.\(^6,7\) To date, megalencephaly associated with NS has been reported only once in medical literature, in an individual affected by megalencephaly-capillary malformation (MCAP) syndrome (OMIM #602501) carrying a 22% mosaic variant in *PIK3CA* (MIM *171834*) and a *PTPN11* germline variant.\(^8\)

Relative megalencephaly has been associated with variants in *SHOC2* gene (MIM *602775*), causing Noonan syndrome-like disorder with loose anagen hair-1 (MIM #607721).\(^9\)

## 2 | CASE HISTORY

We report on a child who came to our attention because of the presence of increased nuchal translucency (3.3 mm) in the first trimester of pregnancy. Second trimester morphology ultrasound revealed right pyelocaliectasis and single umbilical artery. Third trimester ultrasound showed the presence of polydramnios (Amniotic Fluid Index 22 cm) and fetal macrocephaly (head circumference >95% centile). Fetal brain MRI detected enlargement of periencephalic liqueoral spaces and mild lateral ventricular asymmetry. Fetal echocardiography registered the presence of supraventricular extrasystoles. Further prenatal ultrasounds revealed ascites and hepatosplenomegaly, and confirmed the presence of abnormal fetal biometric parameters (head circumference >95% centile and abdominal circumference >95% centile). The patient was born to non-consanguineous and healthy parents after a spontaneous pregnancy at 39 weeks of gestation by cesarean section. Family history was silent. At birth, she was appropriate for gestational age (AGA), with a birth weight of 3.63 kg, her head circumference was 37.3 cm (>97%), and her length was 54 cm (>97% centile). Apgar scores were 9 and 10 at 1 and 5 min after birth, respectively. At birth, she presented minor facial anomalies: hypertelorism, low-set ears, posterior cleft palate, long fingers and toes, single umbilical artery, and axial hypotonia.

Subsequent abdominal ultrasound examinations showed the presence of splenomegaly without pyloleclectasis. Brain MRI, performed at nearly 2 months of age, revealed moderately increased brain volume, mild lateral ventricular asymmetry, thinned dysmorphic corpus callosum, and dysmorphic aspects of brain stem (Figure 1).

Electrocardiographic screening for long QT syndrome demonstrated the presence of Wolf–Parkinson–White Syndrome, treated with beta-blockers and anti-arrhythmic drugs.

Soon after birth, severe leukocytosis appeared (43,950/ mm\(^3\)), although further blood examinations revealed normal leucocyte count at 9 months of age.

With regard to psychomotor development, at 10 months of age she was able to smile in the interaction with the adult, vocalize, turn her head to follow objects, reach out and grasp them, and sit with anterior support. However, she presented reduced eye movement coordination and lower extremities hypotonia.

We evaluated again the child when she was 16 months old. Physical examination showed macrocephaly, frontal bossing, sparse eyebrows, hypertelorism, exophthalmos, depressed nasal root, low-set and posteriorly rotated ears, ligamentous laxity, and hyposthenia (Figure 2). Her weight was 11.3 kg (75° centile), her length was 72.5 cm (5° centile), and her occipital-frontal circumference was 53 cm (>97% centile). She was able to babble and to walk with adult support.

## 3 | INVESTIGATIONS

### 3.1 | WES analysis

Genomic DNA was extracted from peripheral blood samples of proband and parents using standard procedures. The exonic regions and flanking splice junctions of the genome were captured using the Clinical Research Exome v.2 kit (Agilent Technologies). Sequencing was done on a NextSeq500 Illumina system with 150 bp paired-end reads. Reads were aligned to human genome build GRCh37/UCSC hg19 and analyzed for sequence variants using a custom-developed analysis tool.\(^14\) Additional sequencing technology and variant interpretation protocol have been previously described.\(^14\) Coverage on target for the proband was \(\geq 10\times\) for 98% with a mean coverage of 203×.

### 3.2 | Targeted sequencing

The Ion AmpliSeq Custom Panel of the 21 genes involved in the PI3K/AKT/mTOR pathway (i.e., *PIK3R1, PIK3R2, PIK3CA, PTEN, PDK1, PDK2, KRAS, AKT1, AKT2, AKT3, RICTOR, MAPKAP1, MLST8, MTO, IRS1, GAB1, GAB2,*...
FIGURE 1  MR imaging examination performed at nearly 2 months of age. Coronals (A, D) and axials (C, E) TSE T2-weighted images and sagittal (B) 3D-TFE T1-weighted image. MR showed (A) a moderately increased brain volume, (B) a thin and dysmorphic corpus callosum (arrow heads) and a small pons (asterisk). (C) Mild asymmetry of the lateral ventricles with squared off left ventricular frontal horn (arrow). Increased (D) binocular and (E) interocular distances. Abbreviations: TSE, turbo spin echo; 3D-TFE, three-dimensional turbo field echo.

FIGURE 2  Photographs of the patient at birth (A, B), at the age of 10 months (C), and at the age of 16 months (D). Note, macrocephaly, frontal bossing, sparse eyebrows, hypertelorism, exophthalmos, depressed nasal root, and low-set and posteriorly rotated ears.
THEM4, MAPK8I1, PTPN11, and RAPTOR) was used according to our previous report.\textsuperscript{15}

3.3 Alignment

Data analysis was performed using the Torrent Suite Software v5.0.4 (Life Technologies). Reads were aligned to the hg19 human reference genome from the UCSC Genome Browser (http://genome.ucsc.edu/) and to the BED file designed using Ion AmpliSeq Designer. Alignments were visually verified with the software Alamut® v2.13 (Interactive Bio software).

3.4 Coverage analysis

The mean average read depth and the percentage of reads mapping on the ROI out of the total number of reads (reads on target) were calculated using the Coverage Analysis plugin (Torrent Suite v5.0.4 software, Life Technologies). For each sample, the percentage of ROI with a minimum coverage of 100x was calculated using the amplicon coverage matrix file.

3.5 Variant analysis

Variant calling was performed with the Variant Caller plugin configured with somatic high stringency parameters. Variants were annotated using the Ion Reporter 5.14.1.0 software (https://ionreporter.lifetechologies.com/ir/).

Common single nucleotide variants (minor allele frequency [MAF] > 5%), exonic synonymous variants, and intronic variants were removed from the analysis, while exonic nonsynonymous, splice site, and loss-of-function variants were analyzed.

The sequence analysis software Alamut® v2.13 (Interactive Bio software) was used to interpret variants.

Online databases, including dbSNP (database the single nucleotide polymorphism database), 1000 Genomes, ClinVar, EXAC (exome aggregation consortium), COSMIC (catalog of somatic mutations in cancer), and ESP (exome sequencing project), were used. The pathogenicity prediction programs such as PolyPhen2, SIFT, Mutation Taster, and splice prediction programs were used to evaluate variants not previously described.

4 RESULTS

The karyotype and the array-CGH analysis from chorionic villus sampling (CVS) showed a normal female profile. Fast trio-based whole exome sequencing (WES) analysis, performed when the patient was in very critical condition, detected a previously reported de novo heterozygous NRAS missense variant (NM_002524.4:c.34G > A; p.Gly12Ser) in 9 days (REF: Cirstea et al [2010] Nat Genet; ClinVar; Variation ID 177778).

We performed targeted deep sequencing of 21 selected genes belonging to the PI3K/AKT/mTOR pathway on saliva sample. No pathogenic variants were found.

5 DISCUSSION

To date, only few studies regarding the perinatal findings in Noonan syndrome have been described in medical literature. In the prenatal period, the most common clinical characteristics that have been reported are lymphatic anomalies, such as increased nuchal translucency, pleural effusions, cystic hygroma, and hydrops fetalis. Long bones <5° centile have been described in almost half of cases. Other common prenatal findings in NS are as follows: polyhydramnios, macrosomia (defined as abdominal circumference >90th centile), occipital-frontal circumference >90th centile, and congenital heart defects. Arrhythmias have been reported only once in medical literature.

Regarding neonatal findings, cardiovascular anomalies, such as structural heart defects, valvular dysplasia, hypertrophic cardiomyopathy are the most common features. Lymphatic dysplasia, birth weight and occipital-frontal circumference >90th centile, renal defects, and hypotonia are anomalies frequently described in infants affected by NS.\textsuperscript{10}

During the prenatal period, our patient showed specific findings of NS: increased nuchal translucency, right caliectasis, polyhydramnios, abdominal circumference, and occipital-frontal circumference >90th centile, hydrops fetalis.

At birth, other anomalies became evident, such as length >97th centile, long fingers and toes, megalencephaly, and Wolff–Parkinson–White syndrome (WPW). Those characteristics are not typical of Noonan syndrome. Wolff–Parkinson–White syndrome has never been associated with Noonan syndrome.

WPW syndrome is characterized by the presence of an accessory atrioventricular pathway, which bypasses the normal atrioventricular conduction. This pathway leads to a ventricular pre-excitation, causing palpitations, syncope, ventricular fibrillation, and even sudden cardiac death.\textsuperscript{11,12}

Recently, variants in the PI3K-AKT pathway have been described to be associated with overgrowth syndromes (PIK3CA-Related Overgrowth Spectrum, PROS), such as Megalencephaly-capillary malformation-polymicrogyria syndrome (MCAP, MIM # 602501) and Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome-1.
(MPPH, # MIM 603387), but also Congenital lipomatous asymmetric overgrowth, epidermal naevi, skeletal and spinal anomalies syndrome (CLOVES, # MIM 612918), as well as hemimegalencephaly and isolated macroadactyly. For this reason, the analysis of a panel of genes involved in the PI3K/AKT/mTOR pathway was performed from a saliva sample of our patient and the gene panel testing came back negative. Since PROS shows phenotypic variability and it is mostly caused by somatic PIK3CA mutations, it could be important to follow the child over time, in order to monitor the onset of other signs related to PROS. It could be interesting to perform the analysis of a panel of genes involved in the PI3K-AKT pathway in other tissues as well. Indeed, it cannot be excluded that PIK3CA-mutant cells might exert growth-promoting effects on adjacent or distant cells by signal propagation of secreted biomolecules or exosomes.

**AUTHOR CONTRIBUTIONS**
All the authors have contributed equally.

**ACKNOWLEDGMENTS**
We thank the patients’ parents to participate to this study. This work has been generated within the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability (ERN-ITHACA) (EU Framework Partnership Agreement ID: 3HP-HFP-ERN-01-2016/739516).

**CONFLICT OF INTEREST**
All authors declare no competing interests.

**DATA AVAILABILITY STATEMENT**
None.

**CONSENT**
Written informed consent was obtained from the parents of the patient in order to publish this report in accordance with the journal’s patient consent policy.

**ORCID**
Benedetta Beltrami https://orcid.org/0000-0001-6304-0681
Daria Carmela Loconte https://orcid.org/0000-0002-4578-0165
Maria Iascone https://orcid.org/0000-0002-4707-212X
Maria Francesca Bedeschi https://orcid.org/0000-0002-6723-9177

**REFERENCES**
1. Roelofs RL, Janssen N, Wingbermühle E, Kessels RP, Egger JI. Intellectual development in Noonan syndrome: a longitudinal study. Brain Behav. 2016;6(7):e00479. doi:10.1002/brb3.479
2. Yart A, Edouard T. Noonan syndrome: an update on growth and development. Curr Opin Endocrinol Diabetes Obes. 2018;25(1):67-73. doi:10.1097/MED.0000000000000380
3. Altmüller F, Lissewski C, Bertola D, et al. Genotype and phenotype spectrum of NRAS germline variants. Eur J Hum Genet. 2017;25(7):823-831. doi:10.1038/ejhg.2017.65
4. El Bouchikhi I, Belhassan K, Moufid FZ, et al. Noonan syndrome-causing genes: molecular update and an assessment of the mutation rate. Int J Pediatr Adolesc Med. 2016;3(4):133-142. doi:10.1016/j.ijpmam.2016.06.003
5. Shaw AC, Kalidas K, Crosby AH, Jeffery S, Patton MA. The natural history of Noonan syndrome: a long-term follow-up study. Arch Dis Child. 2007;92(2):128-132. doi:10.1136/adc.2006.104547
6. Pavone P, Praticò AD, Rizzo R, et al. A clinical review on megalencephaly: a large brain as a possible sign of cerebral impairment. Medicine (Baltimore). 2017;96(26):e6814. doi:10.1097/MD.0000000000006814
7. Pirozzi F, Nelson B, Mirzaa G. From microcephaly to megalencephaly: determinants of brain size. Dialogues Clin Neurosci. 2018;20(4):267-282. doi:10.31887/DCNS.2018.20.4/gmirzaa
8. Döcker D, Schubach M, Menzel M, et al. Germline PTEN11 and somatic PIK3CA variant in a boy with megalencephaly-capillary malformation syndrome (MCAp) pure coincidence? Eur J Med Genet. 2015;23(3):409-412. doi:10.1016/j.ejmg.2014.118
9. Gripp KW, Zand DJ, Demmer L, et al. Expanding the SHOC2 mutation associated phenotype of Noonan syndrome with loose anagen hair: structural brain anomalies and myelofibrosis. Am J Med Genet A. 2013;161A(10):2420-2430. doi:10.1002/ajmg.a.36098
10. Myers A, Bernstein JA, Brennan ML, et al. Perinatal features of the RASopathies: Noonan syndrome, cardiofaciocutaneous syndrome and Costello syndrome. Am J Med Genet A. 2014;164A(11):2814-2821. doi:10.1002/ajmg.a.36737
11. De Castro RL Jr, de Alcantara Lima N, da Costa Lino DO, Bannon SF. Concealed Wolff-Parkinson-White Syndrome revealed by acute coronary syndrome. Ann Noninvasive Electrocardiol. 2019;25(5):e12735. doi:10.1111/anec.12735
12. Miyamoto L. Molecular pathogenesis of familial Wolff-Parkinson-White syndrome. J Med Invest. 2018;65(1.2):1-8. doi:10.2152/jmi.65.1
13. Keppler-Noreuil KM, Rios JJ, Parker VE, et al. PIK3CA-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. Am J Med Genet A. 2015;167A(2):287-295. doi:10.1002/ajmg.a.36836
14. Pezzani L, Marchetti D, Cereda A, et al. Atypical presentation of pediatric BRAF RASopathy with acute encephalopathy. Am J Med Genet A. 2018;176(1):2869-2872. doi:10.1002/ajmg.a.36739
15. Locente DC, Grossi V, Bozzao C, et al. Molecular and Functional Characterization of Three Different Postzygotic Mutations in PIK3CA-Related Overgrowth Spectrum (PROS) Patients: Effects on PI3K/AKT/mTOR Signaling and Sensitivity to PIK3 Inhibitors. PLoS ONE. 2015;10(4):e0123092. doi:110.1371/journal.pone.0123092.

How to cite this article: Beltrami B, Cerasani J, Consales A, et al. Prenatal overgrowth and polydramnios: Would you think about Noonan syndrome? Clin Case Rep. 2022;10:e06256. doi: 10.1002/ccr3.6256