Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2026**; 53(9): 52097
https://doi.org/10.31083/CEOG52097

Original Research

Prenatal Ultrasound Phenotypes, Genetic Causes, and Inheritance Characteristics of Fetal Noonan Syndrome in Jiaxing

Xiaojia Wu[1], Jianmei Gu[1], Li Wang[1], Ping Tang[1,*]

[1]Department of Fetal Medicine, Jiaxing Maternity and Children Health Care Hospital, 314009 Jiaxing, Zhejiang, China
*Correspondence: phoenix@zjxu.edu.cn (Ping Tang)
Academic Editor: Michael H. Dahan
Submitted: 20 March 2026 Revised: 14 April 2026 Accepted: 6 May 2026 Published: 10 September 2026

Abstract

Background: Noonan syndrome (NS) is a rare autosomal dominant RASopathy characterized by high phenotypic and genetic heterogeneity. Prenatal diagnosis remains challenging due to nonspecific ultrasound (US) abnormalities and limited sensitivity of conventional genetic testing. Data on prenatal US phenotypes, genetic etiology, and inheritance characteristics of fetal NS in Jiaxing region are insufficient. This study aimed to explore the clinical value of whole exome sequencing (WES) in prenatal diagnosis of NS. **Methods:** A retrospective analysis was conducted on 5 cases of prenatally diagnosed NS at the Jiaxing Maternity and Children's Health Care Hospital from January 2020 to January 2025. All fetuses underwent single nucleotide polymorphism (SNP)-array to exclude chromosomal abnormalities and copy number variations (CNVs). WES was performed on fetal amniotic fluid DNA and parental peripheral blood DNA, and candidate pathogenic variants were verified by Sanger sequencing. Variant pathogenicity was classified according to the 2015 American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) Standards and Guidelines combined with the Association for Clinical Genomic Science (ACGS) specifications. Standardized US biometry (percentiles and Z-scores) was used to characterize fetal abnormalities, and the correlation between prenatal US phenotypes and genotypes was analyzed. **Results:** The principal standardized US abnormalities identified across the 5 fetuses included nuchal cystic hygroma (1/5, 20%), choroid plexus cyst (2/5, 40%), macrocephaly (>95th percentile, 2/5, 40%), polyhydramnios (>95th percentile, 4/5, 80%), and fetal growth restriction (FGR; <10th percentile, 1/5, 20%), with all fetuses presenting multiple US abnormalities. Pathogenic or likely pathogenic missense variants were detected in all cases via WES, achieving a 100% diagnostic rate (5/5); pathogenic variants accounted for 60% (3/5), and the likely pathogenic variant detection rate was 40% (2/5). Four core rat sarcoma-mitogen-activated protein kinase (RAS-MAPK) pathway genes were involved (*PTPN11*, *KRAS*, *SOS1*, *RAF1*), corresponding to NS types 1, 3, 4, and 5, respectively. *PTPN11* exhibited the highest variant frequency in this cohort (2/5, 40%, 95% confidence interval [CI]: 5.2%–94.8%). Familial co-segregation analysis confirmed 4 cases (80%) as *de novo* mutations and 1 case (20%) as maternally inherited from an asymptomatic mother. Specific US-genotype correlations were observed in this regional cohort. **Conclusions:** In fetuses from the Jiaxing region with prenatal US abnormalities such as nuchal cystic hygroma, macrocephaly, and polyhydramnios (per International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) 2020 guidelines), as well as negative SNP-array results, NS should be highly suspected. WES was able to successfully identify the genetic etiology and variant inheritance pattern of prenatal NS in this small cohort, and the tiered diagnostic workflow of SNP-array combined with WES and Sanger sequencing has important etiological diagnostic value for such fetuses. This study clarifies the regional US and genetic characteristics of prenatal NS in the Jiaxing region, providing a clinical reference for local prenatal screening, genetic diagnosis, and genetic counseling. Due to the small sample size, the results are exploratory and descriptive in nature, and future large-sample multicenter studies are warranted for validation.

Keywords: Noonan syndrome; prenatal diagnosis; whole exome sequencing; fetal inheritance; ultrasound phenotype; genotype-phenotype correlation; RAS-MAPK pathway; SNP-array

1. Introduction

Noonan syndrome (NS) is a relatively rare autosomal dominant RASopathy with an estimated incidence of 1/1000–1/2500 live births, and a prevalence of 0.04%–0.1% in the general population [1,2,3]. The condition is characterized by genetic and phenotypic heterogeneity, affecting multiple organ systems, including the cardiovascular system, skeletal system, gastrointestinal tract, and may result in hearing and visual impairment [4]. The pathogenesis of NS is closely associated with germline variants in genes encoding key components or regulatory factors of the mitogen-activated protein kinase (RAS-MAPK) signaling pathway, with more than 20 pathogenic genes identified to date, including *PTPN11*, *KRAS*, *SOS1*, and *RAF1* [5,6].

Prenatal diagnosis of NS remains a clinical challenge due to the lack of specific fetal phenotypic manifestations. Fetal NS is usually manifested by non-specific ultrasonic abnormalities such as increased nuchal translucency (NT), nuchal cystic hygroma, fetal edema, cardiovascular abnormalities, macrocephaly, and polyhydramnios. Chromosomal microarray analysis [e.g., single nucleotide polymorphism (SNP)-array] can only exclude chromosomal numer-

ical and structural abnormalities as well as copy number variations (CNVs); however, it cannot identify monogenic variants underlying NS, leading to diagnostic challenges in fetuses with abnormal ultrasound (US) findings and negative chromosomal detection results.

Whole exome sequencing (WES) has been increasingly applied in prenatal genetic diagnosis in recent years, as most disease-causing variants are located in protein-coding exon regions. WES enables efficient sequencing of all exonic regions of the genome, detection of potential pathogenic variants associated with genetic diseases, and identification of novel variant genes, making it a powerful diagnostic tool for rare, genetically heterogeneous conditions such as NS [7,8]. Although several published studies have reported the use of WES for the genetic diagnosis of prenatal NS, the correlation between prenatal US phenotypes and genotypes may differ across populations. At present, limited regional clinical data are available regarding prenatal NS diagnosis in the Jiaxing region.

This retrospective study is the first to systematically analyze the clinical data of 5 prenatally diagnosed NS cases at the Jiaxing Maternity and Children's Health Care Hospital from 2020 to 2025. We aimed to clarify the regional US and genetic characteristics of prenatal NS in the Jiaxing region, verify the clinical utility of a tiered genetic diagnostic workflow (SNP-array → WES → Sanger sequencing validation) for prenatal NS, and explore the specific genotype-phenotype correlations. This study addresses the lack of regional clinical data on prenatal NS diagnosis and provides a targeted reference for local prenatal screening, genetic diagnosis, and genetic counseling, thereby contributing significantly to the improvement of regional prenatal care standards.

2. Materials and Methods

2.1 Study Subjects

A retrospective analysis was performed on the clinical data of 5 cases with a prenatal diagnosis of NS at the Jiaxing Maternity and Children's Health Care Hospital from January 2020 to January 2025. Maternal age ranged from 27–39 years. Prenatal findings included 1 case of NT thickening (4–9 mm) at 11–12 weeks of gestation, 2 cases of macrocephaly (>95th percentile) and polyhydramnios (>95th percentile) after 31^{+0} weeks of gestation, 1 case of fetal growth restriction (FGR, <10th percentile), and 1 case with a history of spontaneous abortion. Amniocentesis was performed in all cases for genetic testing, and following definitive diagnosis of NS and comprehensive genetic counseling, all pregnancies were terminated.

Inclusion criteria were as follows: (1) Fetuses with abnormal prenatal US findings, defined according to the ISUOG (2020) (e.g., nuchal cystic hygroma, macrocephaly >95th percentile, polyhydramnios >95th percentile); (2) Fetuses with negative SNP-array results to exclude chromosomal numerical or structural abnormalities and CNVs; (3) Fetuses diagnosed as NS according to the Clinical Practice Guidelines for Noonan Syndrome (2020) (Chinese Medical Association) [9] via comprehensive analysis of WES results, Sanger sequencing validation, familial co-segregation analysis, and clinical phenotypes; (4) Complete clinical, US, genetic testing, and family data available for analysis; and (5) Pregnant women who provided written informed consent for genetic testing and amniocentesis.

Exclusion criteria were as follows: (1) Fetuses with positive SNP-array results (chromosomal abnormalities/CNVs); (2) Fetuses with other monogenic diseases confirmed by genetic testing; (3) Incomplete US, genetic testing, or family data; (4) Pregnant women with severe maternal conditions (e.g., severe hypertension, diabetes with poor control) that may affect fetal development; and (5) Fetuses with isolated ultrasonic abnormalities without multiple system involvement.

This retrospective study was conducted in accordance with the Declaration of Helsinki and approved by the Prenatal Diagnosis Ethics Committee of Jiaxing Maternity and Children's Health Care Hospital (Approval No.: 2025-Y-115; Approval Date: January 15, 2025). All participants received pre-test genetic counseling and provided written informed consent prior to any invasive procedure and genetic testing. Post-diagnosis genetic counseling included explanation of NS clinical phenotypes (including multiple organ system involvement, short stature, and potential congenital heart defects) [2,10,11], genetic etiology, variant inheritance pattern (*de novo* or maternally inherited), and recurrence risk assessment (2%–5% for *de novo* variants and 50% for autosomal dominant inherited mutations). For the maternally inherited case, the asymptomatic mother received further long-term follow-up and genetic counseling recommendations for subsequent pregnancies.

2.2 Experimental Methods

2.2.1 DNA Extraction

Amniocentesis was performed for pregnant women from all 5 families to collect amniotic fluid samples, and peripheral blood samples were obtained from both parents in each case. Genomic DNA was extracted from all collected samples using a commercial DNA extraction kit (Tiangen Biochemical Technology Co., Ltd., Beijing, China) in strict accordance with the manufacturer's instructions. The purity and concentration of extracted genomic DNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and qualified samples (OD260/280 = 1.8–2.0) were stored at –20 °C for subsequent experimental use.

2.2.2 SNP-Array Analysis

Genomic DNA extracted from amniotic fluid samples was subjected to SNP-array analysis using the Affymetrix CytoScan 750K array (Affymetrix, Santa Clara, CA, USA) following the manufacturer's standard protocol. The raw

data were processed and analyzed using Chromosome Analysis Suite (ChAS) software (Affymetrix, Inc., Santa Clara, CA, USA); the exact software version was not documented in the original experimental records to detect chromosomal numerical and structural abnormalities as well as CNVs, with a resolution of ~50 kb for CNVs and 100 kb for loss of heterozygosity (LOH), so as to preliminarily exclude chromosomal conditions in fetuses.

2.2.3 Whole Exome Sequencing (WES)

WES was performed on qualified genomic DNA samples from fetal amniotic fluid and parental peripheral blood by BGI Co., Ltd. (Shenzhen, Guangdong, China). Briefly, 1 μg of genomic DNA was fragmented into 150–200 bp fragments by ultrasonic shearing, followed by end repair, A-tailing, and adapter ligation with index adapters to construct sequencing libraries. The exonic regions were captured using a SureSelect Human All Exon V6 kit (Agilent Technologies, Santa Clara, CA, USA), which covers ~59 Mb of the human exome and includes 20,965 protein-coding genes. Captured libraries were sequenced on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) with 150 bp paired-end reads, an average sequencing depth of ≥100× for the target regions, and with ≥98% of the target regions covered by ≥20× reads.

2.2.4 Data Analysis

Sequenced reads were aligned to the University of California, Santa Cruz (UCSC) hg19 human reference genome using the Burrows-Wheeler Aligner-MEM (BWA-MEM; Broad Institute of MIT and Harvard, Cambridge, MA, USA); the exact software version was not documented in the original laboratory records. Duplicate reads were removed using Picard tools. Base quality recalibration, single nucleotide variant (SNV), insertion-deletion (INDEL) calling, and genotype detection were subsequently performed using the Genome Analysis Toolkit (GATK, v4.2.6.0; Broad Institute of MIT and Harvard, Cambridge, MA, USA) following the GATK Best Practices pipeline. Exon-level CNV detection was implemented using ExomeDepth software with reference to 100 normal control samples.

Subject information was entered into the analysis system, and variant annotation was conducted by querying major population databases (1000 Genomes Project, ExAC, and gnomAD) and disease-related databases (Online Mendelian Inheritance in Man (OMIM), ClinVar, and Human Gene Mutation Database (HGMD)), combined with bioinformatic pathogenicity prediction tools (Sorting Intolerant From Tolerant (SIFT), Polyphen2, MutationTaster, and Combined Annotation-Dependent Depletion (CADD)). Strict variant filtering and pathogenicity assessment were subsequently performed with the following variant filtering criteria: (1) High-frequency variants with a minor allele frequency (MAF) >0.01 in population databases were excluded; (2) only coding region variants and splice site variants were retained, while benign variants in non-splice-site intronic regions and untranslated regions (UTRs) were excluded; (3) for missense variants, only those predicted to be damaging by at least two independent bioinformatic tools were retained; and (4) variants were annotated for their association with NS to identify candidate pathogenic variants. Pathogenicity rating of candidate variants was finally performed in accordance with the 2015 American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) Standards and Guidelines for the Interpretation of Sequence Variants, combined with the clinical interpretation specifications of the Association for Clinical Genomic Science (ACGS), and classified into 5 categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign.

2.2.5 Sanger Sequencing Validation

Based on the high-throughput WES results, Sanger sequencing was then performed to validate the candidate pathogenic variant sites detected in fetuses and their parental genetic samples. Specific primers were designed for each potential pathogenic variant site using Primer3 software (v0.4.0; Whitehead Institute for Biomedical Research, Cambridge, MA, USA) for polymerase chain reaction (PCR), and primer sequences were synthesized by Sangon Biotech (Shanghai, China). PCR amplification was performed in a 25 μL reaction system containing 12.5 μL of 2× Taq PCR MasterMix, 1 μL of each primer (10 μmol/L), 2 μL of genomic DNA (50 ng/μL), and 8.5 μL of ddH_2O. The PCR reaction conditions were: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55–60 °C for 30 s, 72 °C for 30 s; and a final extension at 72 °C for 10 min. PCR amplification products were purified using a PCR Product Purification Kit (Tiangen Biochemical Technology Co., Ltd., Beijing, China), and Sanger sequencing was conducted on the purified products using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The original primer sequences were not archived for this retrospective study; PCR amplification was performed using laboratory-developed conditions. Sequencing results were aligned to standard reference sequences in GenBank using BLAST software to confirm the precise variant sites, and familial co-segregation analysis was further performed to determine the inheritance pattern of each variant (*de novo* or inherited).

2.2.6 Tiered Prenatal Genetic Diagnostic Workflow

A tiered prenatal genetic diagnostic workflow was adopted for all fetuses with abnormal US findings in this study, which was as follows: SNP-array screening → WES detection → Sanger sequencing validation → familial co-segregation analysis → pathogenicity rating. Briefly, all fetuses first underwent SNP-array analysis to exclude chro-

mosomal abnormalities and CNVs; for fetuses with negative SNP-array results, WES was simultaneously performed on fetal amniotic fluid DNA and parental peripheral blood DNA to identify candidate pathogenic variants associated with fetal abnormal phenotypes. The identified candidate variants were then validated by Sanger sequencing to confirm the variant sites, and familial co-segregation analysis was conducted to determine the variant origin (*de novo* or inherited). Finally, variant pathogenicity was rated according to the 2015 ACMG/AMP guidelines combined with ACGS specifications, and subsequent comprehensive diagnosis of NS was performed by integrating fetal US phenotypes, genetic testing results, variant pathogenicity rating, and familial analysis results [12].

2.3 Observation Parameters

(1) Standardized US and magnetic resonance imaging (MRI) findings: US abnormalities from all fetuses were described using standardized biometry data (percentiles and Z-scores) according to ISUOG guidelines (2019) [13], and US and MRI findings were clearly distinguished; (2) WES test results: Pathogenic genes, chromosomal locus, transcript ID, variant site, exon region, variant type, and ACMG classification of the 5 NS fetuses were recorded; (3) Familial co-segregation analysis results: The inheritance pattern of variants (*de novo* or maternally inherited) was confirmed, and the phenotypic status of variant carriers was recorded; (4) Genotype-phenotype correlation: The correlation between different pathogenic gene mutations and fetal US phenotypes was analyzed; and (5) WES diagnostic efficiency parameters: The diagnostic rate, pathogenic variant detection rate, and likely pathogenic variant detection rate of WES for prenatal NS were calculated.

3. Results

3.1 Standardized US and MRI Findings

All fetal US biometry data were compared with gestational age-matched reference ranges, and abnormalities were defined according to the ISUOG Practice Guidelines for Fetal Ultrasound Examination (2020). Macrocephaly was defined as head circumference >95th percentile for gestational age, polyhydramnios as amniotic fluid index (AFI) >95th percentile, FGR as estimated fetal weight (EFW) <10th percentile, and mild renal pelvis dilatation (Grade I) as anteroposterior diameter 4–9 mm. US and MRI findings were clearly distinguished for each case, with MRI only performed for Case 4 due to unclear intracranial US findings. All cases presented multiple systemic US abnormalities, and the detailed standardized findings were as follows:

Case 1: A 30-year-old pregnant woman, Gravida 1, Para 0 (G1P0), presented at 12^{+0} weeks of gestation. US findings at 12^{+0} weeks of gestation included fetal nuchal cystic hygroma (8 mm in thickness); at 19^{+0} weeks of gestation included bilateral ventricular choroid plexus cysts (3 mm in diameter), and suspected marginal cord insertion. No MRI examination was performed.

Case 2: A 27-year-old pregnant woman, G1P0, presented at 31^{+0} weeks of gestation. US findings: included bilateral extracerebral space widening (Z-score = 2.3, >95th percentile), fetal septum pellucidum widening (3 mm, >95th percentile), biparietal diameter and head circumference >97th percentile for gestational age defined as macrocephaly, femur and humerus length <5th percentile for gestational age defined as skeletal dysplasia, suspected persistent right umbilical vein, and polyhydramnios (AFI = 32 cm, >95th percentile). No MRI examination was performed.

Case 3: A 33-year-old pregnant woman, G1P0, presented at 32^{+0} weeks of gestation. US findings included fetalchoroid plexus cysts (2 mm in diameter), fetal head circumference >95th percentile for gestational age defined as macrocephaly, and polyhydramnios (AFI = 30 cm, >95th percentile). No MRI examination was performed.

Case 4: A 39-year-old pregnant woman, G5P1, presented at 32^{+0} weeks of gestation. US findings included unclear fetal septum pellucidum, bilateral renal pelvis dilatation (Grade I, anteroposterior diameter 6 mm for both sides), and polyhydramnios (AFI = 34 cm, >95th percentile). MRI findings included a small fetal septum pellucidum and normal corpus callosum morphology with no structural abnormality.

Case 5: A 31-year-old pregnant woman, G2P1, presented at 32^{+0} weeks of gestation. US findings included FGR (EFW <10th percentile for gestational age), polyhydramnios, with shortened long bones (femur/tibia length <5th percentile), and microcephaly (head circumference <5th percentile). No MRI examination was performed.

All typical standardized US and MRI abnormal manifestations observed in the 5 NS fetuses described above are visually displayed in Fig. 1, including nuchal cystic hygroma, macrocephaly, choroid plexus cysts, intracranial structural abnormalities, fetal growth restriction, and renal pelvis dilatation.

3.2 WES Test Results, Sanger Sequencing Validation, and ACMG/AMP Classification

WES test achieved a 100% diagnostic rate (5/5) for NS in this regional cohort, with a 60% pathogenic variant detection rate (3/5) and a 40% likely pathogenic variant detection rate (2/5) for fetuses with abnormal US and negative SNP-array results. Combined with Sanger sequencing validation and familial co-segregation analysis, all identified variants exhibited missense mutations in core RAS-MAPK pathway genes, corresponding to NS type 1, 3, 4, and 5, respectively. Variant pathogenicity was rated strictly in accordance with the 2015 ACMG/AMP Standards and Guidelines for the Interpretation of Sequence Variants and combined with ACGS clinical specifications, with clear pathogenicity evidence and gene-phenotype cor-

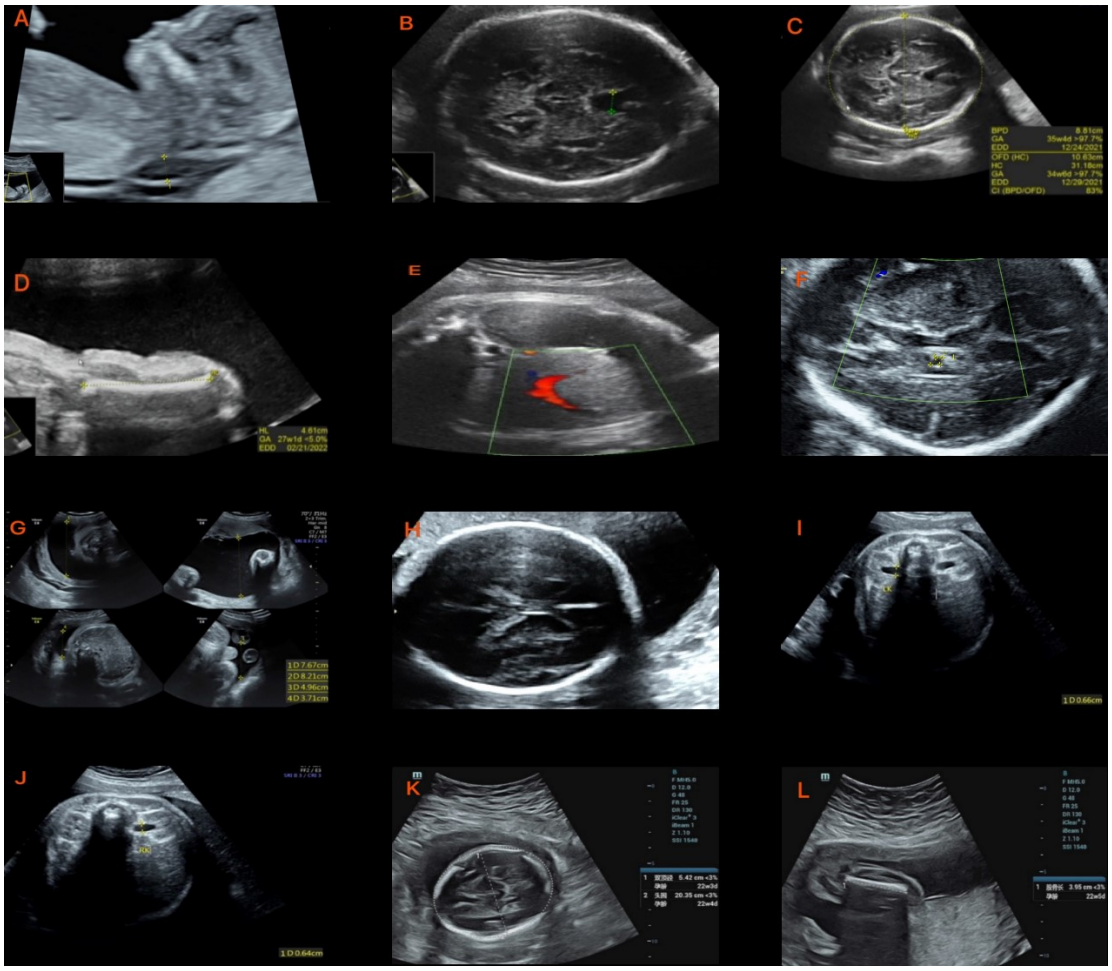

Fig. 1. Representative standardized US and MRI findings in 5 fetuses with NS. (A) Case 1: US: nuchal cystic hygroma (12^{+0} weeks, thickness: 8 mm). (B) Case 2: US: widened septum pellucidum (31^{+0} weeks, 3 mm, Z-score = 2.3). (C) Case 2: US: macrocephaly (biparietal diameter and head circumference >97th percentile). (D) Case 2: US: skeletal dysplasia (femur length <5th percentile). (E) Case 2: US: suspected persistent right umbilical vein. (F) Case 3: US: choroid plexus cyst (32^{+0} weeks, diameter: 2 mm). (G) Case 3: US: polyhydramnios (32^{+0} weeks, AFI = 30 cm). (H) Case 4: US: unclear septum pellucidum. (I,J) Case 4: US: bilateral Grade I renal pelvis dilatation. (K) Case 5: US: FGR with microcephaly. (L) Case 4: MRI: small septum pellucidum with normal corpus callosum. All US abnormalities were defined per ISUOG 2020 guidelines; AFI, amniotic fluid index; FGR, fetal growth restriction; US, ultrasound; MRI, magnetic resonance imaging; NS, Noonan syndrome; ISUOG, International Society of Ultrasound in Obstetrics and Gynecology.

relations annotated for all variants (Table 1). No frameshift, splice site, or nonsense mutations were detected in this cohort.

PTPN11 (2/5, 40%, 95% confidence interval [CI]: 5.2%–94.8%) presented the highest mutation frequency in this cohort, with two distinct missense mutations in the EX7/CDS7 region (Case 1: c.784C>T, p.Leu262Phe; Case 5: c.794G>A, p.Arg265Gln) [14,15,16]. Case 1 was classified as likely pathogenic, and Case 5 was classified as pathogenic; both variants were confirmed as *de novo* mutations with no identical variants detected in parental peripheral blood samples. The two *PTPN11*-mutated fetuses presented typical NS-related US abnormalities, including nuchal cystic hygroma and choroid plexus cysts, showing a specific genotype-phenotype correlation.

Case 2 carried a *de novo* likely pathogenic *KRAS* missense mutation (c.108A>G, p.Ile36Met) in the EX2/CDS1 region (1/5, 20%, 95% CI: 0.5%–79.5%), corresponding to NS type 3. The fetus presented with severe multiple US abnormalities, including macrocephaly, skeletal dysplasia, persistent right umbilical vein, and polyhydramnios, consistent with the clinical phenotypic characteristics of *KRAS*-associated NS (craniocerebral and skeletal dysplasia).

Case 3 carried a pathogenic *SOS1* missense mutation (c.1642A>C, p.Ser548Arg) in the EX10/CDS10 region (1/5, 20%, 95% CI: 0.5%–79.5%) [17], comprising the only maternally inherited variant in the present cohort. Familial co-segregation analysis confirmed that the asymptomatic mother carried the identical variant with no obvious NS-related clinical phenotypes, reflecting the phenotypic het-

Table 1. WES results of 5 fetuses with NS.

Case No.	Mutated gene	Chromosomal locus	Transcript ID	Variant site (c.DNA/p.Protein)	Exon region	Variant type	ACMG classification	Pathogenicity evidence	Gene-phenotype correlation	Related NS subtype (OMIM ID)	Inheritance pattern
1	*PTPN11*	chr12:112910775	NM_002834.3	c.784C>T(p.Leu262Phe)	EX7/CDS7	Missense mutation	Likely pathogenic	(1) *De novo* mutation; (2) MAF <0.01 (gnomAD); (3) Damaging (SIFT/Polyphen2); (4) Associated with NS (OMIM)	Nuchal cystic hygroma +Choroid plexus cyst	Type 1 (163950)	*De novo*
2	*KRAS*	chr12:25398211	NM_033360.2	c.108A>G(p.Ile36Met)	EX2/CDS1	Missense mutation	Likely pathogenic	(1) *De novo* mutation; (2) MAF <0.01 (gnomAD); (3) Damaging (MutationTaster/CADD); (4) Associated with NS (OMIM)	Macrocephaly + skeletal dysplasia + polyhydramnios	Type 3 (609942)	*De novo*
3	*SOS1*	chr2:39249927	NM_005633.3	c.1642A>C(p.Ser548Arg)	EX10/CDS10	Missense mutation	Pathogenic	(1) Maternally inherited (asymptomatic carrier); (2) MAF <0.01 (gnomAD); (3) Damaging (4 tools); (4) Confirmed NS-associated variant (ClinVar)	Choroid plexus cyst + polyhydramnios (no FGR)	Type 4 (610733)	Maternal inheritance (asymptomatic mother)
4	*RAF1*	chr3:12641216	NM_002880.3	c.1082G>C(p.Gly361Ala)	EX10/CDS9	Missense mutation	Pathogenic	(1) *De novo* mutation; (2) MAF <0.01 (gnomAD); (3) Damaging (SIFT/Polyphen2/CADD); (4) RAF1 hotspot variant for NS	Intracranial abnormality + renal pelvis dilatation + severe polyhydramnios	Type 5 (611553)	*De novo*
5	*PTPN11*	chr12:112910785	NM_002834.3	c.794G>A(p.Arg265Gln)	EX7/CDS7	Missense mutation	Pathogenic	(1) *De novo* mutation; (2) MAF <0.01 (gnomAD); (3) Damaging (4 tools); (4) Classic NS-associated *PTPN11* variant	Choroid plexus cyst + FGR	Type 1 (163950)	*De novo*

Table Notes: (1) MAF, minor allele frequency; (2) ACMG classification based on 2015 ACMG/AMP guidelines + ACGS clinical specifications; (3) Irrelevant disease listings are removed; only NS subtypes and direct genotype-phenotype correlations are included; (4) All variants were verified by Sanger sequencing, with familial co-segregation analysis for inheritance pattern confirmation.

OMIM, Online Mendelian Inheritance in Man; WES, whole exome sequencing; SIFT, Sorting Intolerant From Tolerant; CADD, Combined Annotation-Dependent Depletion; ACMG, American College of Medical Genetics and Genomics; AMP, Association for Molecular Pathology; ACGS, Association for Clinical Genomic Science.

erogeneity of NS. This fetus presented only with choroid plexus cysts and polyhydramnios without FGR or skeletal dysplasia, consistent with the reported phenotypic characteristics of *SOS1*-mutated NS type 4 (rarely associated with short stature).

Case 4 harbored a *de novo* pathogenic *RAF1* missense mutation (c.1082G>C, p.Gly361Ala) in the EX10/CDS9 region (1/5, 20%, 95% CI: 0.5%–79.5%) [18,19], corresponding to NS type 5. The fetus presented with intracranial structural abnormalities (poorly defined septum pellucidum), mild renal pelvis dilatation, and severe polyhydramnios; no cardiovascular structural abnormalities were detected by prenatal US. These findings expand the prenatal phenotypic spectrum of *RAF1*-associated NS in this regional cohort.

3.3 Summary Statistics of Key Clinical and Genetic Variables

Descriptive statistical analysis was performed on key variables across the 5 NS fetuses (frequency + percentage), including standardized US abnormalities, variant gene distribution, variant classification, and inheritance pattern (Table 2). All fetuses presented multiple systemic US abnormalities (5/5, 100%), with polyhydramnios was observed in 4/5 cases, and macrocephaly in 2/5 cases, followed by choroid plexus cysts (2/5, 40%). *De novo* mutations were the dominant inheritance pattern (4/5, 80%), and missense mutations were the only variant type detected (5/5, 100%). Pathogenic variants accounted for 60% (3/5) of all identified variants, and likely pathogenic variants accounted for 40% (2/5).

4. Discussion

4.1 Specific Genotype-Phenotype Correlations in This Regional NS Cohort

Consistent with previously reported global data [1,2,5], the 4 core pathogenic genes identified in this study (*PTPN11*, *KRAS*, *SOS1*, and *RAF1*) are all established RAS-MAPK pathway genes, with *PTPN11* exhibiting the highest variant frequency in this small regional cohort (2/5, 40%, 95% CI: 5.2%–94.8%). This frequency is consistent with the reported global *PTPN11* variant rate (~50%) in NS patients [2,16,20], further confirming the predominant role of *PTPN11* in the genetic etiology of NS. The *PTPN11* gene is located at chromosomal region 12q24 and encodes the non-receptor protein tyrosine phosphatase SHP-2, which regulates multiple intracellular signaling pathways and is involved in cell growth, differentiation, and proliferation [14,15]. Missense mutations in the EX7/CDS7 region, detected in both Case 1 and Case 5, can lead to constitutive activation of SHP-2, loss of autoinhibition, and abnormal upregulation of the RAS-MAPK pathway, ultimately inducing NS. Of the two *PTPN11*-variant fetuses in this cohort, one presented with typical nuchal cystic hygroma and choroid plexus cysts, a specific genotype-phenotype correlation not previously emphasized in regional studies. This finding may provide a targeted screening clue for local prenatal US diagnosis: NS should be suspected when a fetus presents with the combination of nuchal cystic hygroma and choroid plexus cysts in the setting of negative SNP-array results. This finding may provide a targeted screening clue for local prenatal US diagnosis: NS should be suspected when a fetus presents with the combination of nuchal cystic hygroma and choroid plexus cysts in the setting of negative SNP-array results.

The *KRAS* variant frequency in this cohort (20%, 95% CI: 0.5%–79.5%) was higher than the reported global clinical rate (~2%) in NS patients [2,5]. Given the insufficient statistical power of this study (n = 5), no Fisher's exact test was performed to assess this discrepancy, and this elevated local frequency should not be considered representative of the general NS population. The potential reasons for this discrepancy include regional genetic heterogeneity of NS, selection bias as this study only included fetuses with multiple severe US abnormalities, which may be more likely to carry *KRAS* mutations, and the small sample size. The *KRAS* gene is a member of the RAS family located at chromosomal region 12p12.1, encoding a guanosine triphosphatase (GTPase) that regulates the RAS-MAPK pathway [5,6]. The c.108A>G (p.Ile36Met) variant identified in this study is a gain-of-function mutation that results in persistent activation of the RAS-MAPK pathway. The *KRAS*-variant fetus presented with severe macrocephaly, skeletal dysplasia, and polyhydramnios, consistent with the reported clinical characteristics of NS type 3, which is associated with craniocerebral and skeletal dysplasia with severe multisystem involvement [2]. These findings suggest that *KRAS* variants are associated with severe fetal structural US abnormalities and warrant heightened clinical attention in local prenatal screening.

The *SOS1*-variant case in this cohort, in which the variant was maternally inherited from an asymptomatic carrier, represents a typical manifestation of NS phenotypic heterogeneity [1,2,5]. *SOS1* is the second most frequently implicated gene in NS [2,20], located at chromosomal region 2p22.1, and encodes a membrane-bound guanine nucleotide-binding protein that regulates cell growth and differentiation through modulation of RAS-MAPK pathway signaling [5,6]. The fetus in this case presented solely with choroid plexus cysts and polyhydramnios without FGR or skeletal dysplasia, consistent with published reports that *SOS1* variants are rarely associated with short stature in NS patients [2,17]. In addition, *SOS1*-variant NS patients are prone to pulmonary valve stenosis but rarely present atrial septal defect, which is a specific phenotypic characteristic of this subtype [2]. The discovery of this asymptomatic maternal carrier has important local clinical implications for genetic counseling, as this suggests that NS has hidden genetic transmission in local families, and WES combined with familial co-segregation analysis can identify

Table 2. Summary statistics of key clinical and genetic variables in 5 fetuses with NS.

Key variable category	Subcategory	Frequency (n)	Percentage (%)	Supplementary quantitative note
Standardized US abnormalities	Polyhydramnios	4	80	AFI >95th percentile for gestational age
	Macrocephaly/intracranial abnormality	2	40	Head circumference >95th percentile/structural intracranial changes
	Choroid plexus cyst	2	40	Diameter 2–3 mm, bilateral/unilateral
	Nuchal cystic hygroma	1	20	Thickness 8 mm (12^{+0} weeks)
	FGR	1	20	EFW <10th percentile + shortened long bones
	Renal pelvis dilatation (Grade I)	1	20	Anteroposterior diameter 6 mm (bilateral)
	Skeletal dysplasia	1	20	Long bone length <5th percentile
	Multiple US abnormalities	5	100	≥2 systemic abnormalities in all cases
Mutated gene distribution	*PTPN11*	2	40	95% CI: 5.2%–94.8%
	KRAS	1	20	95% CI: 0.5%–79.5%
	SOS1	1	20	95% CI: 0.5%–79.5%
	RAF1	1	20	95% CI: 0.5%–79.5%
Variant type	Missense mutation	5	100	No other mutation types detected
ACMG variant classification	Pathogenic	3	60	Including 2 *de novo*, 1 maternal inheritance
	Likely pathogenic	2	40	Both *de novo* mutations
Inheritance pattern	*De novo* mutation	4	80	No parental variant carriage
	Maternal inheritance	1	20	Asymptomatic mother as variant carrier

Table Notes: (1) All US abnormalities defined per ISUOG 2020 guidelines; (2) 95% CI calculated by exact binomial distribution (suitable for small sample size).
EFW, estimated fetal weight.

hidden carriers and provide accurate recurrence risk assessment for secondary pregnancies in local families.

The *RAF1*-variant fetus in this study presented with intracranial structural abnormalities, mild renal pelvis dilatation, and severe polyhydramnios, with no cardiac structural abnormalities detected by prenatal US. *RAF1* is located at the chromosomal region 3p25, encoding a serine-threonine kinase that serves as a key component of the RAF-MEK-ERK cascade within the RAS-MAPK pathway [5,6,18]. *RAF1* variants are known to be closely associated with hypertrophic cardiomyopathy in NS patients, with the principal hotspot mutation positions at Ser259 and Ser621 [2,18,19]. The absence of cardiac abnormalities in the present case expands the prenatal phenotypic spectrum of *RAF1*-associated NS in the Jiaxing region, suggesting that *RAF1* variants may also lead to multiple fetal systemic US abnormalities beyond cardiac defects during the prenatal period. These regional phenotypic data contribute to the broader understanding of prenatal manifestations of *RAF1*-associated NS and provide a novel reference for prenatal phenotypic prediction in this NS subtype.

Notably, all 5 fetuses in this cohort presented with multiple systemic US abnormalities (100%), with polyhydramnios was observed in 4/5 cases, and macrocephaly in 2/5 cases. This finding is consistent with international prenatal NS series and suggests that multiple systemic US abnormalities represent a key prenatal phenotypic clue for NS, both in the Jiaxing region and globally. In fetuses with negative SNP-array results and combined polyhydramnios and macrocephaly, NS should be strongly suspected, and WES should be recommended. Furthermore, 80% of variants in this cohort arose *de novo*, which is consistent with the established genetic characteristics of NS, in which *de novo* variants account for a substantial proportion of prenatal cases [1,2].

In addition, 4 of the 5 cases in this cohort harbored *de novo* variants. Although advanced paternal age (≥35 years) is widely recognized as a risk factor for *de novo* autosomal dominant variants, only one father in this series was of advanced paternal age, which precludes any definitive association between advanced paternal age and the occurrence of *de novo* NS variants in this cohort. Further studies with

larger sample sizes are needed to explore this relationship. This underscores that NS prenatal diagnosis in this region cannot rely solely on family genetic history, and that WES should be pursued in fetuses with suspicious US abnormalities regardless of a family history of NS.

4.2 Clinical Utility and Inherent Limitations of WES in Prenatal NS Diagnosis

WES served a central role in etiological diagnosis in this study. In this regional cohort of fetuses with abnormal US findings and negative SNP-array results, WES achieved a 100% detection rate for pathogenic or likely pathogenic variants related to NS. As an established high-throughput sequencing technology, WES enables efficient sequencing of all protein-coding exonic regions of the genome, with the advantages of high detection accuracy (over 99% for exonic variants), broad genomic coverage, and relatively low cost. These characteristics make WES particularly well suited for the prenatal diagnosis of rare monogenic diseases with genetic heterogeneity, such as NS [5,6,7,8,21]. The tiered diagnostic workflow employed in this study, specifically SNP-array screening → WES detection → Sanger sequencing validation → familial co-segregation analysis, yielded a definitive etiological diagnosis in all 5 cases, confirming the clinical utility of this approach in local prenatal diagnosis. In the local clinical setting, this workflow can effectively solve the diagnostic challenge posed by fetuses with abnormal US findings and negative chromosomal detection results, providing a clear genetic basis for prenatal genetic counseling and pregnancy decision-making.

The well-recognized inherent limitations of WES must be acknowledged in the context of prenatal diagnosis. First, WES exhibits coverage gaps in certain exonic regions, such as those with high GC content regions or repetitive sequences, which may lead to missed detection of rare variants. Second, WES has relatively limited sensitivity for detecting CNVs and mosaic variants compared with chromosomal microarray analysis and targeted deep sequencing. Although this study combined WES with ExomeDepth for exon-level CNV detection, large-fragment CNVs (>1 Mb) and low-level mosaic variants (<20%) may still be missed. Third, WES cannot detect variants in non-coding regions, such as promoters, enhancers, and intronic splice sites, that may be associated with NS, and therefore does not provide complete coverage of all potential pathogenic variants. Fourth, WES cannot identify pathogenic variants caused by epigenetic modifications (e.g., DNA methylation) or genomic imprinting abnormalities. In this study, these limitations were mitigated by combining WES with Sanger sequencing validation and familial co-segregation analysis, and no missed variants were identified across the 5 cases. Nevertheless, these limitations should be clearly explained to patients during pre-test genetic counseling in local clinical practice.

Given the small sample size (n = 5) and the selected nature of the cohort, which included only genetically confirmed NS-positive cases the sensitivity, specificity, and positive predictive value of WES for prenatal NS diagnosis could not be calculated in this study. These key diagnostic metrics require verification through future large-sample multicenter studies incorporating NS-negative controls, including fetuses with isolated US abnormalities and negative genetic testing results. Moreover, the 100% diagnostic rate achieved by WES in this study is applicable only to this small regional cohort of genetically confirmed NS-positive fetuses and cannot be extrapolated to the general prenatal population.

5. Limitations

5.1 Study Limitations

This study presents several limitations that should be acknowledged, and all conclusions are exploratory and descriptive in nature and only applicable to the Jiaxing region with the same clinical setting. First, the small sample size (n = 5), attributable to the low incidence of NS, resulted in wide 95% CIs for variant frequencies and insufficient statistical power for comparative analysis with globally reported data; no binomial test or Fisher's exact test was performed [2,5]. The genetic and phenotypic characteristics identified in this study require validation by larger-sample regional studies [11,22,23]. Second, the absence of long-term follow-up data, as all pregnancies were terminated after diagnosis, precluded confirmation of postnatal clinical phenotypic manifestations and prevented correlation analysis between prenatal US findings and postnatal clinical manifestations [2,10,11]. Third, selection bias in the study population, as only fetuses presenting with severe multiple US abnormalities and negative SNP-array results were included; fetuses with isolated US abnormalities or mild NS manifestations were not captured, which may lead to an overestimation of the association between severe US abnormalities and NS in this region. Fourth, the absence of external validation, as the results derive exclusively from a single center (Jiaxing Maternity and Children's Health Care Hospital); multicenter studies across the Yangtze River Delta region are needed to further validate the regional US and genetic characteristics of NS. Fifth, the limited detection range of WES, which, as mentioned above, cannot detect non-coding region variants, large-fragment CNVs, or low-level mosaic variants, potentially resulting in missed diagnoses in NS cases harboring these types of pathogenic variants [7,8,21].

5.2 Clinical Implications for Local Prenatal Diagnosis

Despite the abovementioned limitations, this study presents important clinical implications for the prenatal diagnosis of NS in the Jiaxing region and surrounding areas. First, it clarifies the key prenatal US clues for NS in the local region: multiple systemic US abnormalities (especially

polyhydramnios + macrocephaly), nuchal cystic hygroma, and choroid plexus cysts (with negative SNP-array results). These clues can help local obstetricians and fetal medicine specialists identify high-risk fetuses and recommend further genetic testing in a timely manner. Second, it verifies the clinical value of the tiered genetic diagnostic workflow (SNP-array → WES → Sanger validation) for prenatal NS, which can be recommended as the standard diagnostic pipeline for local fetuses with suspicious NS manifestations [7,8,21]. This workflow can improve the diagnostic accuracy and efficiency of prenatal NS, as well as reduce the rate of missed diagnosis or misdiagnosis. Third, it identifies the specific genotype-phenotype correlations of NS in the local area, which can provide a targeted reference for local genetic counseling (e.g., recurrence risk assessment, phenotypic prediction) [2,5,6]. For example, *PTPN11* mutation is associated with nuchal cystic hygroma and choroid plexus cysts, and *KRAS* mutation is associated with severe multiple system structural abnormalities [2,14,15,16]. Fourth, it emphasizes the importance of familial co-segregation analysis. For NS-related variants detected by WES, parental genetic testing is necessary to clarify the inheritance pattern (*de novo* or maternally inherited) and identify hidden asymptomatic carriers, which is crucial for accurate genetic counseling and secondary pregnancy guidance [1,2]. Fifth, it highlights the value of standardized US biometry in prenatal NS screening. Using percentiles and Z-scores to describe fetal abnormalities according to ISUOG guidelines can improve the consistency and accuracy of prenatal US diagnosis, and facilitate the identification of NS-related US manifestations [13].

6. Conclusions

In fetuses presenting with multiple systemic prenatal US abnormalities (e.g., nuchal cystic hygroma, macrocephaly, polyhydramnios, and choroid plexus cysts), as defined by the ISUOG 2020 guidelines, in the setting of negative SNP-array results, NS should be highly suspected in clinical practice in the Jiaxing region. In this cohort of fetuses selected for US anomalies and negative SNP-array results, WES achieved a 100% detection rate for known pathogenic variants of NS. This tiered diagnostic workflow demonstrates clear clinical value in this case series by establishing a molecular etiology and enabling precise genetic counseling. However, as this study included only genetically confirmed NS cases, the 100% detection rate reflects the efficiency of variant detection in this preselected cohort and should not be interpreted as evidence of general clinical utility in unselected prenatal populations. These results should be regarded as exploratory in nature and require validation in larger, unselected cohorts [5,6,7,8,21]. This study systematically characterizes the regional US and genetic characteristics of prenatal NS in the Jiaxing region, demonstrating that *PTPN11* is the most frequently implicated gene, multiple systemic US abnormalities represent the key prenatal phenotypic clue, and *de novo* mutations constitute the predominant inheritance pattern [1,2,16,20]. These findings provide a targeted clinical reference for local prenatal NS screening, genetic diagnosis, and genetic counseling, and are of great significance for improving the level of regional prenatal care standards and genetic diagnosis.

All conclusions are exploratory and descriptive in nature due to the small sample size and single-center design, and cannot be extrapolated to the general prenatal population or other geographic regions. Future large-sample, multicenter, prospective studies are needed to: (1) validate the regional US and genetic characteristics of NS in the Yangtze River Delta region; (2) calculate the sensitivity, specificity, and positive predictive value of WES for prenatal NS diagnosis [7,8,21]; (3) analyze the correlation between prenatal US phenotypes and postnatal clinical manifestations of NS [2,10,11]; and (4) further optimize the prenatal screening and diagnostic workflow for NS in the broad Chinese population [9,11,22]. In addition, attention should be paid to the inherent technical limitations of WES in clinical practice, and pre-test genetic counseling should fully inform patients of these limitations as well as the exploratory nature of WES results for prenatal NS diagnosis.

Availability of Data and Materials

All data reported in this paper will also be shared by the lead contact upon reasonable request.

Author Contributions

XW, PT, and JG designed this study. XW, JG, and LW conducted the research, including sample collection, experimental operation, and data collection. PT provided assistance in the design of the research plan and suggestions for the overall writing approach of the manuscript. XW and JG analyzed the experimental data and drafted the initial manuscript. All authors have contributed to the editing and revision of the manuscript, read and approved the final manuscript, and participated sufficiently in the work to take public responsibility for the content.

Ethics Approval and Consent to Participate

This retrospective study was carried out in accordance with the guidelines of the Declaration of Helsinki. It was approved by the Prenatal Diagnosis Ethics Committee at the Jiaxing Maternity and Children Health Care Hospital, China (Ethic Approval Number: 2025-Y-115; Approval Date: January 15, 2025). All participants received pre-test genetic counseling and provided signed informed consent before amniocentesis and genetic testing.

Acknowledgment

We sincerely appreciate Jiaxing Maternity and Children Health Care Hospital for providing technical assistance in sample sequencing and clinical data collection. We

also appreciate the constructive comments from the reviewers, which greatly improved the quality of this manuscript. Finally, we would like to express our gratitude to all the research participants for their collaboration and support.

Funding

This research was funded by the Research on People's Livelihood Science and Technology Innovation of Jiaxing Science and Technology Program Project, grant number: 2022AD30093.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Johnston JJ, van der Smagt JJ, Rosenfeld JA, Pagnamenta AT, Alswaid A, Baker EH, et al. Autosomal recessive Noonan syndrome associated with biallelic LZTR1 variants. Genetics in Medicine: Official Journal of the American College of Medical Genetics. 2018; 20: 1175–1185. https://doi.org/10.1038/gim.2017.249

[2] Roberts AE, Allanson JE, Tartaglia M, Gelb BD. Noonan syndrome. Lancet (London, England). 2013; 381: 333–342. https://doi.org/10.1016/S0140-6736(12)61023-X

[3] Yart A, Edouard T. Noonan syndrome: an update on growth and development. Current Opinion in Endocrinology, Diabetes and Obesity. 2018; 25: 67–73. https://doi.org/10.1097/MED.0000000000000380

[4] Romano AA, Allanson JE, Dahlgren J, Gelb BD, Hall B, Pierpont ME, et al. Noonan syndrome: clinical features, diagnosis, and management guidelines. Pediatrics. 2010; 126: 746–759. https://doi.org/10.1542/peds.2009-3207

[5] Tajan M, Paccoud R, Branka S, Edouard T, Yart A. The RASopathy Family: Consequences of Germline Activation of the RAS/MAPK Pathway. Endocrine Reviews. 2018; 39: 676–700. https://doi.org/10.1210/er.2017-00232

[6] Tidyman WE, Rauen KA. Pathogenetics of the RASopathies. Human Molecular Genetics. 2016; 25: R123–R132. https://doi.org/10.1093/hmg/ddw191

[7] Gahl WA, Markello TC, Toro C, Fajardo KF, Sincan M, Gill F, et al. The National Institutes of Health Undiagnosed Diseases Program: insights into rare diseases. Genetics in Medicine. 2012; 14: 51–59. https://doi.org/10.1038/gim.0b013e318232a005

[8] Li RJ, Li CY, Huang DQ, Tan JF, Zhang HQ. Application of whole exome sequencing technology in fetuses with cardiac rhabdomyoma. Journal of Xiangnan University (Medical Sciences). 2025; 27: 31–34. https://doi.org/10.16500/j.cnki.1673-498x.2025.01.008

[9] Clinical practice guidelines for Noonan syndrome, Li X, Wang X, Wang J, Fu L, Luo X, et al. Clinical practice guidelines for Noonan syndrome. Chinese Journal of Medical Genetics. 2020; 37: 324–328. https://doi.org/10.3760/cma.j.issn.1003-9406.2020.03.017

[10] Romano AA, Dana K, Bakker B, Davis DA, Hunold JJ, Jacobs J, et al. Growth response, near-adult height, and patterns of growth and puberty in patients with noonan syndrome treated with growth hormone. The Journal of Clinical Endocrinology and Metabolism. 2009; 94: 2338–2344. https://doi.org/10.1210/jc.2008-2094

[11] Li X, Wen T, Feng BY, Wang XM. Growth and development patterns of Noonan syndrome and advances in the treatment of short stature. Chinese Journal of Contemporary Pediatrics. 2025; 27: 33–38. https://doi.org/10.7499/j.issn.1008-8830.2409047

[12] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in Medicine. 2015; 17: 405–424. https://doi.org/10.1038/gim.2015.30

[13] Salomon LJ, Alfirevic Z, Da Silva Costa F, Deter RL, Figueras F, Ghi T, et al. ISUOG Practice Guidelines: ultrasound assessment of fetal biometry and growth. Ultrasound in Obstetrics & Gynecology. 2019; 53: 715–723. https://doi.org/10.1002/uog.20272

[14] Qiu W, Wang X, Romanov V, Hutchinson A, Lin A, Ruzanov M, et al. Structural insights into Noonan/LEOPARD syndrome-related mutants of protein-tyrosine phosphatase SHP2 (PTPN11). BMC Structural Biology. 2014; 14: 10. https://doi.org/10.1186/1472-6807-14-10

[15] Martinelli S, Nardozza AP, Delle Vigne S, Sabetta G, Torreri P, Bocchinfuso G, et al. Counteracting effects operating on Src homology 2 domain-containing protein-tyrosine phosphatase 2 (SHP2) function drive selection of the recurrent Y62D and Y63C substitutions in Noonan syndrome. The Journal of Biological Chemistry. 2012; 287: 27066–27077. https://doi.org/10.1074/jbc.M112.350231

[16] Sznajer Y, Keren B, Baumann C, Pereira S, Alberti C, Elion J, et al. The spectrum of cardiac anomalies in Noonan syndrome as a result of mutations in the PTPN11 gene. Pediatrics. 2007; 119: e1325–1331. https://doi.org/10.1542/peds.2006-0211

[17] Lepri F, De Luca A, Stella L, Rossi C, Baldassarre G, Pantaleoni F, et al. SOS1 mutations in Noonan syndrome: molecular spectrum, structural insights on pathogenic effects, and genotype-phenotype correlations. Human Mutation. 2011; 32: 760–772. https://doi.org/10.1002/humu.21492

[18] Kobayashi T, Aoki Y, Niihori T, Cavé H, Verloes A, Okamoto N, et al. Molecular and clinical analysis of RAF1 in Noonan syndrome and related disorders: dephosphorylation of serine 259 as the essential mechanism for mutant activation. Human Mutation. 2010; 31: 284–294. https://doi.org/10.1002/humu.21187

[19] Razzaque MA, Nishizawa T, Komoike Y, Yagi H, Furutani M, Amo R, et al. Germline gain-of-function mutations in RAF1 cause Noonan syndrome. Nature Genetics. 2007; 39: 1013–1017. https://doi.org/10.1038/ng2078

[20] Zenker M, Edouard T, Blair JC, Cappa M. Noonan syndrome: improving recognition and diagnosis. Archives of disease in childhood. 2022; 107: 1073–1078. https://doi.org/10.1136/archdischild-2021-322858

[21] LaDuca H, Farwell KD, Vuong H, Lu HM, Mu W, Shahmirzadi L, et al. Exome sequencing covers >98% of mutations identified on targeted next generation sequencing panels. PLoS ONE. 2017; 12: e0170843. https://doi.org/10.1371/journal.pone.0170843

[22] Stuurman KE, Joosten M, van der Burgt I, Elting M, Yntema HG, Meijers-Heijboer H, et al. Prenatal ultrasound findings of rasopathies in a cohort of 424 fetuses: update on genetic testing in the NGS era. Journal of Medical Genetics. 2019; 56: 654–661. https://doi.org/10.1136/jmedgenet-2018-105746

[23] Zenker M, Lehmann K, Schulz AL, Barth H, Hansmann D, Koenig R, et al. Expansion of the genotypic and phenotypic spectrum in patients with KRAS germline mutations. Journal of medical genetics. 2007; 44: 131–135. https://doi.org/10.1136/jmg.2006.046300