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Original Article

Clinical and molecular spectrum of RASopathies in a pediatric cohort: A single-center retrospective study

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ABSTRACT

Objective: Noonan syndrome (NS) and related RASopathies are genetically heterogeneous disorders caused by dysregulation of the RAS/MAPK signaling pathway and are characterized by overlapping clinical features, including distinctive craniofacial appearance, growth impairment, congenital heart defects (CHD), and variable neurodevelopmental involvement. Comprehensive molecular characterization is essential for accurate diagnosis and genotype–phenotype correlation. Evaluation of well-characterized single-center pediatric cohorts may provide clinically relevant real-world insight into genotype–phenotype relationships within routine practice. The aim of this study is to delineate genotype–phenotype correlations in a well-characterized pediatric cohort.

Materials and Methods: This retrospective single-center study included pediatric patients between January 2022 and September 2025 with molecularly confirmed diagnosis of NS and related RASopathies. Clinical, laboratory, and imaging findings were reviewed. Targeted next-generation sequencing of RASopathy-associated genes was performed. Detected variants were interpreted according to American College of Medical Genetics and Genomics guidelines, and segregation analysis was conducted when available.

Results: The cohort comprised 20 pediatric patients from 19 unrelated families, with a mean age of 6.1 years. CHD was identified in 80% of patients, most commonly pulmonary valve stenosis (65%), while cardiac involvement was not universal. Short stature was observed in 65% of cases and represented the most frequent reason for referral. Disease-associated variants were identified in nine different genes, with *PTPN11* being the most frequently affected (40%), followed by *LZTR1* (15%), *NF1* (10%), and *RAF1* (10%). Most variants were classified as pathogenic or likely pathogenic, whereas a limited number were categorized as variants of uncertain significance. Integrated interpretation incorporating phenotype–genotype concordance and segregation data supported the potential clinical relevance of selected uncertain variants.

Conclusion: This pediatric cohort highlights the marked clinical and genetic heterogeneity of NS and related RASopathies. The absence of cardiac involvement in some patients underscores the limitations of phenotype-based assessment alone. Comprehensive molecular testing remains critical for accurate diagnosis, refinement of genotype–phenotype correlations, and appropriate long-term management.

Keywords: Genotype–phenotype correlation, genetic testing, MAP kinase signaling system, next-generation sequencing, noonan syndrome, rasopathies

Introduction

The Ras/mitogen activated protein kinase (MAPK) pathway plays an essential role in regulating critical cellular processes such as growth, differentiation, proliferation, aging, and apoptosis. By transducing extracellular mitogenic signals into the cell, this pathway helps coordinate cellular responses that are vital for development and maintaining homeostasis. It consists of a series of protein kinases—RAS, RAF, MEK, and ERK—which interact in a cascade to modulate various cellular functions (1).

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Genetic alterations within components of the Ras/MAPK pathway can disrupt these cellular processes, leading to a range of developmental and physiological anomalies. RASopathies are a group of genetic disorders caused by pathogenic germline mutations in genes involved in the Ras/MAPK signaling pathway (2, 3). These conditions are clinically diverse and can manifest with a wide range of symptoms, complicating diagnosis and management. Common features include CHDs, craniofacial anomalies, growth delays, cognitive impairments, skin abnormalities, and cancer predisposition (4, 5).

Noonan syndrome (NS) is the most common form of RASopathies, with an estimated incidence of approximately 1 in 1,000–2,500 live births. Clinically, NS is defined by a recognizable constellation of findings such as characteristic craniofacial features, postnatal growth impairment, and CHD—most frequently pulmonary valve stenosis (PS), atrial septal defect (ASD), or hypertrophic cardiomyopathy (HCM). Additional manifestations may involve a short or webbed neck, thoracic deformities with superior pectus carinatum and inferior pectus excavatum, cryptorchidism, bleeding diatheses due to coagulation abnormalities, lymphatic dysplasia, and ophthalmologic anomalies (6,7). Several other conditions—including Cardiofaciocutaneous syndrome (CFCS), Costello syndrome (CS), Legius syndrome (LGSS), Neurofibromatosis–Noonan syndrome (NFNS), Noonan-like syndrome with loose anagen hair (NSLH), and Noonan syndrome with multiple lentigines (NSML) formerly known as LEOPARD syndrome—share overlapping clinical and molecular characteristics with NS. Due to these shared features and their common involvement of the Ras/MAPK pathway, these disorders are collectively classified as NS-related disorders (8).

To date, pathogenic variants in numerous genes encoding components or regulators of the Ras/MAPK pathway have been implicated in the molecular etiology of NS and related disorders. These include *PTPN11, SOS1, SOS2, RAF1, RIT1, LZTR1, MAP2K1, MAP2K2, BRAF, KRAS, NRAS, HRAS, SHOC2, CBL, SPRED1, NF1, RRAS2, RASA2, PPP1CB, MAPK1*, and *MRAS* (8,9).

A defining characteristic of RASopathies is their pronounced clinical overlap and phenotypic variability, whereby comparable clinical manifestations may arise from pathogenic variants in different genes, while distinct phenotypic profiles can result from variants affecting the same gene. This observation highlights the inherent complexity of genotype–phenotype correlations across RASopathies, and underscores the limited discriminatory value of clinical findings alone. Accordingly, comprehensive molecular characterization is essential for accurate diagnosis, improved prognostic stratification, and the implementation of individualized clinical monitoring and emerging targeted therapeutic approaches (5).

The aim of this study was to retrospectively analyze the clinical, molecular, and laboratory characteristics of pediatric cases diagnosed with NS and related disorders. This analysis sought to broaden the understanding of the clinical spectrum, evaluate the relationships between genetic variants and phenotypic manifestations, and clarify genotype–phenotype correlations.

Materials and Methods

This study was designed as a retrospective cohort study conducted at Samsun Training and Research Hospital. Written informed consent for both clinical evaluation and genetic testing was obtained from the parents or legal guardians of all participants, in accordance with the Declaration of Helsinki.

Pediatric patients who were suspected of having NS and related disorders, underwent genetic testing between January 2022 and September 2025, and whose diagnoses were subsequently confirmed by molecular genetic analysis were included in the study.

Demographic and clinical data were retrospectively extracted from patient files and electronic medical records. Collected variables included: age at presentation, sex, presenting symptoms, consanguinity, family history, growth parameters (including height, weight, and head circumference percentiles), craniofacial features, cardiac anomalies, skeletal abnormalities, cutaneous findings, developmental millestones, and involvement of other organ systems. Additionally, laboratory findings, including bleeding parameters, and imaging studies performed for tumor screening were recorded when available.

Short stature was defined as height <–2 SDS, low body weight as weight <–2 SDS, and macrocephaly as head circumference > +2 SDS for age and sex. Relative macrocephaly was defined as a head circumference within the normal range but disproportionately large in relation to height and/or weight. Developmental milestones were assessed using the Denver Developmental Screening Test II (Denver II), and age-appropriate Wechsler Intelligence Scale for Children–Revised (WISC-R), when applicable.

For genetic analysis, genomic DNA was extracted from peripheral blood leukocytes using standard protocols. Targeted sequencing was performed for genes associated with RASopathies, including *BRAF, CBL, HRAS, KRAS, LZTR1, MAP2K1, MAP2K2, NF1, NRAS, PTPN11, RAF1, RASA2, RIT1, SHOC2, SOS1, SOS2,* and *SPRED1*, using hybridization-based capture with the xGen™ DNA Library Prep EZ Kit and related IDT enrichment kits (Integrated DNA Technologies, USA). Genomic DNA was fragmented and enriched according to the manufacturer's instructions, and sequencing libraries were generated accordingly.

Sequencing was performed on Illumina® platforms, achieving a mean coverage depth of 70×–100×, with ≥90% of target regions covered at this depth. Variant calling and primary filtering were conducted after base calling, with low-quality variants excluded during initial filtering steps.

Bioinformatic analysis was carried out using a standardized pipeline. Quality control of raw sequencing data was performed with FastQC, followed by adapter trimming and read filtering using Cutadapt. Reads were aligned to the GRCh37/hg19 human reference genome using the BWA-MEM algorithm. Duplicate reads were marked, base quality score recalibration was applied, and variant calling was performed in accordance with GATK Best Practices. Variants with a read depth <10× were excluded from further analysis.

Variant annotation was performed using the Ensembl Variant Effect Predictor. Only variants located within protein-coding exons and flanking intronic regions (±10 base pairs) were evaluated. Variants were prioritized if they had a minor allele frequency <1% in public population databases, including gnomAD, ExAC, 1000 Genomes, dbSNP, and Kaviar. Previously reported disease-associated variants were assessed using ClinVar, HGMD® Public, Franklin, and VarSome databases.

The potential functional impact of identified variants was evaluated using multiple in silico prediction tools, including SIFT, PolyPhen-2, MutationTaster, MutationAssessor, LRT, CADD, DANN, FATHMM, FATHMM-MKL, MetaSVM, MetaLR, GERP, phyloP, phastCons, and SiPhy. Variant interpretation and classification were performed in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines, taking into account all known inheritance models.

Statistical analysis

Descriptive statistics were used to summarize the data. Continuous variables were expressed as mean and standard deviation (SD), and categorical variables were presented as frequencies and percentages.

Results

Demographic and Clinical Characteristics

A total of 20 pediatric patients from 19 unrelated families were included in the study. The cohort consisted of 11 females (55%) and 9 males (45%). The mean age at evaluation was 6.1 years, ranging from 1 month to 13.5 years.

Parental consanguinity was noted in one patient, and two patients had a parent with phenotypic features suggestive of NS. Prenatal history was unremarkable in most cases; however, polyhydramnios was reported in 2 patients (10%), and intrauterine growth restriction in 1 patient (5%).

The most common reasons for referral for genetic investigation were short stature and congenital heart disease, together accounting for 65% of cases. Short stature was present in 13 patients (65%), and low body weight was observed in seven patients (35%). None of the patients met the criteria for macrocephaly.

Neurodevelopmental milestones were within the normal range in 12 patients (60%), whereas developmental delay of varying severity was observed in 8 patients (40%). Hypotonia was documented in 1 patient (5%).

A typical facial phenotype consistent with NS was observed in 12 of 20 patients (60%). Among the most frequent dysmorphic features, hypertelorism was observed in 19 patients (95%), ptosis in 12 patients (60%), downslanting palpebral fissures in 11 patients (55%), high-arched palate in 7 patients (35%), and short neck in 5 patients (25%). Representative facial phenotypes observed in Noonan syndrome and related disorders are illustrated in Figure 1.

CHD was identified in 16 patients (80%), while 4 patients (20%) had no detectable cardiac anomalies. The most frequent cardiac abnormality was PS, observed in 13 patients (65%). Other cardiac findings included ASD in 6 patients (30%), HCM in 3 patients (15%), ventricular septal defect in 1 patient (5%), and aortic stenosis in 1 patient (5%). Some patients exhibited more than one cardiac anomaly.

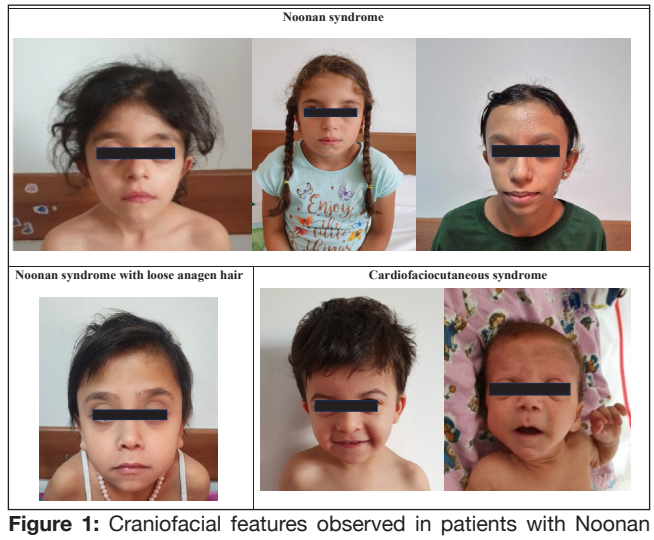


Figure 1: Craniofacial features observed in patients with Noonan syndrome, Noonan syndrome with loose anagen hair, and cardiofaciocutaneous syndrome.

Pectus deformity was identified in 7 patients (35%), including pectus excavatum in 5 patients (25%) and pectus carinatum in 2 patients (10%). Scoliosis was present in 2 patients (10%), and cubitus valgus was observed in 2 patients (10%).

Ectodermal manifestations were common, with café-au-lait macules observed in 6 patients (30%) and lentigines in 2 patients (10%). Deep palmar creases were identified in 3 patients (15%) and hair anomalies in 2 patients (10%). Additionally, a cutaneous vascular malformation was detected in 1 patient (5%).

Cryptorchidism was present in 2 patients (10%), while no other genitourinary anomalies were identified. Ophthalmologic abnormalities were limited to strabismus, observed in 2 patients (10%). Hearing loss was detected in 1 patient (5%). No lymphatic malformations were identified in any of the patients in our cohort. Initial hematologic and oncologic assessments revealed no abnormalities in any patient.

The overall clinical characteristics of the study cohort are summarized in the table I.

Molecular Genetic Findings

A definite molecular diagnosis was established in all 20 patients through integrated clinical and genetic evaluation. Overall, 9 different genes were implicated in the molecular etiology. All detected variants were heterozygous. A total of 19 distinct variants were identified, as the monozygotic twins shared the same *PTPN11* variant. A comprehensive summary of the genetic findings is provided in Table II.

The most frequently affected gene was *PTPN11*, with variants identified in 8 patients (40%). Other genes included *LZTR1* in 3 patients (15%), *NF1* in 2 patients (10%), and *RAF1* in 2 patients (10%). Single patients (5% each) harbored variants in *BRAF, SHOC2, SPRED1, RIT1*, and *MAP2K2*.

Table I: Demographic and clinical characteristics of the study cohort

No	Gender/ Age*	Presenting Symptoms	Height (SDS)	Craniofacial findings	CA	Skeletal findings	Ectodermal findings	DD/ID	Other
1	F/123	DD	-4.5	H, ptosis, high-arched palate, relative macrocephaly	-	-	CALMs, deep palmar and plantar creases, fine and sparse slow-growing hair	+	Strabismus
2	M/92	Short stature	-3.1	H, ptosis, DPF, triangular face	PS, ASD	CV	-	-	-
3	M/3	CA	-0.96	H, ptosis, DPF, short neck	PS, ASD	-	CALMs	-	Cryptorchidism
4	F/5	CA	-1.23	H, high-arched palate	PS	CV	CALMs	-	-
5	M/94	Short stature	-2.34	H, ptosis, epicanthus	HCM	-	-	-	-
6	F/124	Short stature	-3.43	H, DPF, deep philtrum, short neck, and webbed neck	-	PE, scoliosis	-	-	-
7	F/124	Lentigines	-2.09	H, depressed nasal bridge, high-arched palate	PS ASD	-	Multiple lentigines	+	-
8	F/162	Dysmorphism	-2.05	H, ptosis, DPF, triangular face	PS	Scoliosis	-	-	Strabismus
9	F/100	Short stature	-2.1	Depressed nasal bridge, high-arched palate	PS	PE	CALMs	+	-
10	M/2	CA	-0.8	H, ptosis, DPF, epicanthus, depressed nasal bridge, high-arched palate, deep philtrum, pointed chin, low-set ears	PS	PE	Sparse hair, sparse eyebrows, deep palmar creases, loose skin	-	Hearing loss, hypotonia
11	M/120	Short stature	-2.4	H, ptosis, DPF	PS	PE, scoliosis	-	-	-
12	M/1	CA	-1.57	H, ptosis, DPF, dysplastic and low-set ears, brachycephaly, short neck	VSD, ASD, HCM	-	-	-	Cryptorchidism
13	F/98	Short stature	-3.2	H, depressed nasal bridge	-	-	CALMs	-	-
14	M/18	CA	-0.42	H, ptosis, DPF, prominent and broad forehead, deep philtrum, short neck	HCM	-	-	-	-
15	F/6	Short stature	-2.05	H, DPF, epicanthus, high-arched palate	-	None	CALMs	-	-
16	F/103	Dysmorphism	-2.17	H, ptosis, DPF, proptosis	PS	PE	-	+	-
17	M/58	DD	-3.47	H, ptosis, DPF, prominent forehead, relative macrocephaly	PS	Pectus carinatum	Deep palmar creases,	+	-
18	M/24	DD	-1.12	H, ptosis, dysplastic and low-set ears, depressed nasal bridge, prominent forehead	PS	-	-	+	Cutaneous vascular malformation
19	F/124	Lentigines	-2.02	H, depressed nasal bridge, high-arched palate	PS, ASD	None	Multiple lentigines	+	-
20	F/11	CA	0.95	H, prominent forehead, deep philtrum, short neck	PS, AS, ASD	Pectus carinatum	-	-	-

***:** *months,* ***M:** *male,* ***F:** *female,* ***CA:** *Cardiac anomaly* ***DD:** *Developmental delay,* ***H:** *Hypertelorism,* ***DPF:** *Downslanting palpebral fissures,* ***CV:** *Cubitus valgus,* ***PE:** *Pectus excavatum,* ***SDS:** *standard deviation score,* ***PS:** *pulmonary stenosis,* ***ASD:** *atrial septal defect,* ***HCM:** *hypertrophic cardiomyopathy,* ***VSD:** *ventricular septal defect,* ***AS:** *aortic stenosis,* ***CALMs:** *café-au-lait macules,* ***ID:** *Intellectual disability*

Table II: Molecular genetic findings of the study cohort

No	Confirmed Diagnosis	Gene	Nucleotide Change	Aminoacid Change	Zygosity	Variant Type	Variant Classification (ACMG)	Novel/ Known	dbSNP ID	Inheritance
1	NSLH	*SHOC2*	c.4A>G	p.(Ser2Gly)	Het	Missense	P	Known	rs267607048	De novo
2	NS	*PTPN11*	c.317A>C	p.(Asp106Ala)	Het	Missense	P	Known	rs397507517	
3	NS	*PTPN11*	c.184T>G	p.(Tyr62Asp)	Het	Missense	P	Known	rs121918460	
4	NFNS	*NF1*	c.7415del	p.(Pro2472LeufsTer17)	Het	Frameshift	P	Known	rs1597859768	De novo
5	NS	*LZTR1*	c.27dup	p.(Gln10AlafsTer24)	Het	Frameshift	P	Known	rs587777613	
6	NS	*PTPN11*	c.5C>T	p.(Thr2Ile)	Het	Missense	P	Known	rs267606990	
7	NSML	*PTPN11*	c.836A>G	p.(Tyr279Cys)	Het	Missense	P	Known	rs121918456	De novo
8	NS	*LZTR1*	c.742G>A	p.(Gly248Arg)	Het	Missense	P	Known	rs869320686	De novo
9	NFNS	*NF1*	c.3461A>T	p.(Asn1154Ile)	Het	Missense	LP	Known	rs371544233	
10	CFCS	*BRAF*	c.770A>G	p.(Gln257Arg)	Het	Missense	P	Known	rs180177035	De novo
11	NS	*PTPN11*	c.188A>G	p.(Tyr63Cys)	Het	Missense	P	Known	rs121918459	
12	NS	*PTPN11*	c.417G>C	p.(Glu139Asp)	Het	Missense	P	Known	rs397507520	De novo
13	LGSS	*SPRED1*	c.304dup	p.(Thr102AsnfsTer7)	Het	Frameshift	P	Known	rs1555391053	
14	NS	*RAF1*	c.769T>C	p.(Ser257Pro)	Het	Missense	LP	Known	rs727505017	De novo
15	NS	*LZTR1*	c.1495G>A	p.(Val499Met)	Het	Missense	VUS	Known	rs773420178	De novo
16	NS	*PTPN11*	c.417G>C	p.(Glu139Asp)	Het	Missense	P	Known	rs397507520	
17	CFCS	*MAP2K2*	c.247G>A	p.(Gly83Ser)	Het	Missense	VUS	Known	rs765755554	Mat
18	NS	*RAF1*	c.761G>A	p.(Arg254Lys)	Het	Missense	VUS	Novel		De novo
19	NSML	*PTPN11*	c.836A>G	p.(Tyr279Cys)	Het	Missense	P	Known	rs121918456	De novo
20	NS	*RIT1*	c.335G>C	p.(Gly112Ala)	Het	Missense	P	Known	rs672601335	Pat

NSLH : *Noonan-like syndrome with loose anagen hair;* ***NS:*** *Noonan Syndrome;* ***NFNS:*** *Neurofibromatosis–Noonan syndrome;* ***NSML:*** *Noonan syndrome with multiple lentigines;* ***CFCS:*** *Cardiofaciocutaneous syndrome;* ***LGSS:*** *Legius syndrome;* ***Het:*** *heterozygous;* ***P:*** *pathogenic;* ***LP:*** *likely pathogenic;* ***VUS:*** *variant of uncertain significance;* ***ACMG:*** *American College of Medical Genetics and Genomics;* ***dbSNP:*** *Single Nucleotide Polymorphism Database;* ***Mat****: maternal;* ***Pat:*** *paternal*

Based on combined clinical and molecular findings, the final diagnoses were NS in 12 patients (60%), NFNS in 2 patients (10%), CFCS in 2 patients (10%), NSML in 2 patients (10%), LGSS in 1 patient (5%), and NSLH in 1 patient (5%).

Regarding variant type, 16 of the 19 variants (84.2%) were missense, while 3 variants (15.8%) were frameshift. According to ACMG classification, 14 variants (73.7%) were pathogenic, 2 (10.5%) were likely pathogenic, and 3 (15.8%) were classified as variant of uncertain significance (VUS). Only one variant was novel, whereas all remaining variants had been previously reported.

Segregation analysis was available for 12 patients; two had a affected parent with suggestive features of NS, while the remaining variants were confirmed to be de novo, and the affected parents were referred for further clinical evaluation.

The three VUS were identified in *LZTR1*, *MAP2K2*, and *RAF1*. The MAP2K2 variant was detected in a patient presenting with a phenotype consistent with CFCS, including short stature, relative macrocephaly, ptosis, hypertelorism, downslanting palpebral fissures, prominent forehead, PS, pectus deformity, and developmental delay. The same variant was also identified in the patient's mother, who exhibited a similar phenotype accompanied by mild intellectual disability. A VUS variant in the *LZTR1* was detected in a patient with typical NS facial features, short stature, and café-au-lait macules and was confirmed to be de novo. A novel VUS in *RAF1* (c.761G>A) was identified in a patient with characteristic NS facial features and PS, and was confirmed to be de novo. Based on the concordance between the clinical phenotype, molecular findings, and the available segregation data, this variant was considered potentially disease-associated.

Discussion

In this study, we evaluated the clinical and molecular characteristics of a pediatric cohort consisting of 20 patients diagnosed with NS and related disorders. In our study cohort, NS constituted the most common diagnostic category, accounting for 60% of cases. However, the clinical spectrum extended beyond classic NS and also included NFNS, CFCS, NSML, LGSS, and NSLH. The most frequently identified genetic etiology was *PTPN11* variants, accounting for 40% of cases, followed by *LZTR1* (15%), *NF1* (10%), and *RAF1* (10%). Overall, variants classified as pathogenic, likely pathogenic, and selected VUS were identified in nine different genes, highlighting the marked genetic heterogeneity of RASopathies (5).

The frequency of *PTPN11* mutations observed in our cohort (40%) is broadly consistent with published data, in which *PTPN11* variants account for approximately half of NS cases (10). In comparison with national data, our findings are closely

aligned with the 50% mutation rate reported in a recent Turkish RASopathy cohort by Alkaya et al. (11), while exceeding the lower frequency (27%) documented in an earlier Turkish series by Şimşek-Kiper et al. (12). From an international perspective, reported *PTPN11* mutation frequencies vary substantially across populations, ranging from 29% in an Indian cohort to 51% in a large Russian series (13,14). Overall, these inter-study differences likely reflect variations in patient selection, cohort size, diagnostic approaches, and population-specific genetic backgrounds.

Notably, no *SOS1* variants were identified in our cohort, despite *SOS1* accounting for approximately 10–20% of Noonan syndrome cases (15,16). Moreover, SOS1 variants have been reported in 19–27% of patients in Turkish cohorts (11,12). This absence is likely attributable to the relatively small sample size, but may also be related to the phenotypic characteristics of our cohort. In the literature, *SOS1* variants are generally associated with a milder clinical phenotype, the presence of ectodermal features (such as keratosis pilaris and curly hair), and, most importantly, a lower frequency of short stature or an association with normal height (17, 18). Therefore, patients harboring *SOS1* mutations, who often exhibit normal stature, may not have been referred for genetic evaluation, as short stature was present in 65% of patients in our cohort.

A particularly notable finding was the relatively high prevalence of *LZTR1* variants, detected in 15% of patients, which exceeds the generally reported prevalence of 4–9% in the literature (19). However, our finding is comparable to the 11.1% reported in a recent Turkish cohort, suggesting that *LZTR1* mutations may be more frequent in the Turkish population than previously estimated in broader international series (11).

In our study, a novel missense variant in *RAF1* (c.761G>A) was identified in a patient exhibiting characteristic NS facial features and PS. This variant is located within the highly conserved region 2 (CR2) domain of the *RAF1* protein, a critical regulatory region that includes the 14-3-3 binding motif. Pathogenic variants clustering in this region, particularly around the Ser257 residue, are known to interfere with the inhibitory binding of 14-3-3 proteins, thereby leading to sustained activation of the Ras/MAPK pathway and a high predisposition to HCM (20). Although the de novo occurrence and the close proximity to known pathogenic clusters strongly support its clinical significance, the variant is currently classified as a VUS. To definitively upgrade its classification according to ACMG guidelines, functional evidence is required. Until such functional evidence is obtained, the variant remains potentially disease-associated based on the strong concordance between the clinical phenotype, molecular findings, and available segregation data.

Patients in our cohort classified as having NFNS presented with café-au-lait macules, PS, and pectus deformity, while lacking classic *NF1* features such as neurofibromas or Lisch nodules. The phenotypic overlap between NS and *NF1* has been well recognized in the literature (4, 21). Given that *NF1* can give rise to distinct clinical presentations, an NFNS overlap diagnosis should be considered in patients with Noonan-like features who do not fully meet diagnostic criteria for *NF1*.

Cardiovascular involvement was observed in 80% of patients, with PS being the most common anomaly (65%). This frequency is consistent with the reported 50–60% prevalence of PS in NS (22). In terms of genotype–phenotype correlations, the strong association between *PTPN11* mutations and PS is well established (23). PS was indeed the predominant cardiac finding among *PTPN11*-positive patients in our cohort, observed in 6 of 8 patients (75%). In contrast, *RAF1* mutations have been reported to be associated with HCM in up to 80–95% of cases (24). Most pathogenic *RAF1* variants cluster within the CR2, particularly around Ser257, and are strongly linked to severe HCM (20, 24). In our cohort, two patients harbored *RAF1* variants, one presenting with HCM and the other with PS. Similarly, *RIT1* variants are known to confer a high risk of HCM (56–75%) in NS patients; however, in our cohort, the *RIT1*-positive patient presented with PS (25,26). While strong genotype–phenotype correlations have been established for certain genes, cardiac manifestations across RASopathies represent a broader and more variable cardiac spectrum. Importantly, in our cohort, four patients had no detectable cardiac abnormalities, underscoring that structural heart disease, although common, is not an obligatory feature of NS, and emphasizing that molecular diagnosis should not be excluded in the absence of cardiac findings.

Genotype–phenotype correlations have been described for growth impairment across NS and related disorders. *PTPN11*-positive individuals are frequently reported to be shorter than NS patients without *PTPN11* variants, whereas normal stature or a lower frequency of short stature has been described in specific subgroups such as *SOS1* and RIT1–related NS (26-28). In contrast, *SHOC2*-related NSLH is consistently associated with prominent ectodermal features and postnatal growth failure, and severe short stature has been repeatedly reported (29,30). Consistent with these gene-specific trends, short stature was the most common reason for referral in our cohort, being most severe in the *SHOC2*-positive patient (height SDS −4.5) and frequent among *PTPN11*-positive patients (6/8, 75%), whereas normal stature was observed in the *RIT1*-positive patient, underscoring both established correlations and the overall heterogeneity of growth outcomes in NS and NS-related disorders.

No malignancies were observed in our cohort. Nevertheless, NS patients—particularly those with *PTPN11* and *KRAS* mutations—are known to have an increased risk of juvenile myelomonocytic leukemia and other hematologic malignancies (31,32). Moreover, *LZTR1* variants have been associated with a predisposition to schwannomatosis (33). These considerations highlight the importance of long-term clinical follow-up in affected patients.

Limitations

Despite its comprehensive molecular characterization, this study has several limitations. The sample size is relatively small (n=20), which limits the ability to generalize the frequency of rare genetic variants or establish definitive genotype–phenotype correlations for less common genes. For instance, the absence of SOS1 or *HRAS* variants in our cohort may be due to the limited number of participants rather than a true absence in the local population. As a single-center retrospective study, there may be an inherent referral bias; patients with more severe or classic clinical presentations

(such as significant short stature or complex cardiac defects) might have been more likely to be referred for genetic testing, potentially excluding those with milder phenotypes. A significant limitation of this study is the absence of functional validation for the identified novel and uncertain variants. Future multi-center studies with larger, longitudinal cohorts are needed to further clarify the regional genetic landscape of RASopathies.

Conclusion

In conclusion, our single-center pediatric cohort highlights the marked clinical and genetic heterogeneity of Noonan syndrome and related RASopathies. Using a targeted multigene panel, a molecular diagnosis was established in all patients, with *PTPN11* as the most frequent cause, followed by *LZTR1*, *NF1*, and RAF1. Integrated interpretation of selected VUS, incorporating phenotype–genotype concordance and segregation data alongside ACMG criteria, proved valuable for resolving diagnostic gray zones. The observed phenotypic variability, including variable cardiac and growth involvement, underscores the limitations of clinical assessment alone. Overall, our findings support comprehensive molecular testing to refine diagnosis, guide surveillance, and enable accurate genetic counseling in NS-spectrum disorders.

Ethics committee approval

This study was conducted in accordance with the Helsinki Declaration Principles. The study was approved by Samsun University Ethics Committee (01.12.2025, reference number: GOKAEK 2025/21/4).

Contribution of the authors

A.S. and O.S. contributed to the conception and design of the study. AS, TKC, EU, ES, and OS. were responsible for data collection and clinical evaluation. AS, MBM, and OS. performed data analysis and interpretation. AS. drafted the initial version of the manuscript. TKC, EU, MBM, ES, and O.S. critically revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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