Molecular Syndromology

Research Article

Mol Syndromol
DOI: 10.1159/000549593

Received: March 31, 2025
Accepted: November 13, 2025
Published online: November 17, 2025

Molecular and Clinical Profiles of Patients with RASopathies: Targeted Next-Generation Sequencing Panel Results and Identification of 14 Novel Disease-Causing Variants

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Keywords
Neurofibromatosis type 1 · Next-generation sequencing · Noonan syndrome · Novel variant · RASopathy

Abstract

Introduction: RASopathies are among the most prevalent genetic syndromes caused by variants in the Ras/MAPK signaling pathway, affecting various systems such as the heart, craniofacial features, skin, musculoskeletal system, hearing, and vision. They can also increase the risk of secondary malignancies. Despite clinical overlaps, distinguishing features are crucial for diagnosis, as different variants lead to distinct clinical implications. This study reviews the molecular and clinical characteristics of RASopathies, focusing on neurofibromatosis type 1 (NF1) and non-NF1 RASopathies. ***Methods:*** The study analyzed 76 patients referred to our outpatient clinic over a 6-year period, all of whom were clinically diagnosed with RASopathy and confirmed in most cases by molecular testing. Patient files, clinical photographs, and laboratory results were reviewed and analyzed. A targeted multigene next-generation sequencing panel test was performed, followed by Sanger sequencing for both confirmation and segregation analysis. Multiplex ligation-dependent probe amplification was conducted in a patient with normal sequence results but strong clinical suspicion, to identify potential deletions. ***Results:*** We identified 44 pathogenic, 25 likely pathogenic variants, and 6 variants of uncertain significance based on American College of Medical Genetics and Genomics (ACMG) criteria. Among these, 14 novel variants were found – 13 in the *NF1* gene and one in *SOS1*. *NF1* variants were present in 51 cases. Additional variants, likely to represent clinically significant findings, were identified in *PTPN11* ($n = 11$), *RAF1* ($n = 4$), *SOS1* ($n = 3$), RIT1 ($n = 3$), *KRAS* ($n = 1$), *NRAS* ($n = 1$), *SOS2* ($n = 1$), and *BRAF* ($n = 1$). Diagnoses included 49 patients with NF1, 21 with Noonan syndrome, 2 with neurofibromatosis-Noonan syndrome, 2 with Noonan syndrome with multiple

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lentigines, and 1 with cardiofaciocutaneous syndrome. Here, 12% of *NF1* variants were located in exon 21, 36% of *PTPN11* variants in exon 3, and 75% of *RAF1* variants in exon 7. ***Conclusion:*** RASopathies have a broad molecular and clinical spectrum, complicating diagnosis and management. Accurate clinical correlation and molecular analysis are essential, as different RASopathy syndromes can result from variants in the same genes, while the same syndrome may arise from different genetic alterations. This study identifies novel variants and emphasizes the need for precise diagnostic approaches in these complex disorders.

Introduction

RASopathies are a group of genetic disorders characterized by germline variants in the Ras/mitogen-activated protein kinase (Ras/MAPK) signaling pathway, which plays a crucial role in cellular growth, differentiation, and survival [1, 2]. Variants in the genes within the Ras/MAPK) signaling pathway cause RASopathy disorders. The most well-characterized RASopathies include neurofibromatosis type 1 (NF1) (von Recklinghausen disease), Noonan syndrome (NS), neurofibromatosis-NS, Noonan syndrome with multiple lentigines (NSMLs) (formerly LEOPARD), NS with loose anagen hair, Costello syndrome, Legius syndrome (NF1-like syndrome), cardiofaciocutaneous syndrome, and capillary malformation-arteriovenous malformation syndrome. Each of these disorders is associated with unique phenotypic presentations; however, they share an underlying etiology rooted in the dysregulation of Ras/MAPK signaling. Common features are characterized by cardiac anomalies, growth deficiencies, homeostatic defects, dermatological findings, musculoskeletal problems, vision and/or hearing defects, distinctive facial features, cognitive impairment, and susceptibility to secondary malignancies.

The general incidence of RASopathies is estimated to be 1 in ~1,000 newborns, making them one of the most common congenital malformation disorders [3, 4]. When examining these disorders individually, NF1 occurs at a frequency of approximately 1 in 3,000 births, while NS has a reported incidence of 1 in 1,000 to 2,500 live births [5].

In this study, patients who received a clinical diagnosis of RASopathy, as well as those in whom molecular testing identified variants potentially consistent with their clinical presentation, were analyzed for genotypic and phenotypic characteristics. We compared the demographic data with existing literature, assessed the impact of potential hotspot variants and genotypes on clinical findings, and presented 14 novel variants.

Materials and Methods

Subjects

The clinical data of 76 patients (98 total including family members) who were diagnosed with RASopathy and consulted our outpatient clinic over a 6-year period were analyzed. In the study, we analyzed patients who had received a clinical diagnosis of RASopathy, together with those in whom molecular testing detected variants that could be considered consistent with their clinical manifestations, in order to characterize both genotypic and phenotypic features. However, cases lacking molecular analysis were excluded. Informed consent forms were obtained from the legal guardians of patients under the age of 18, as well as from the patients themselves if they were aged 18 and over.

Sample Preparation and Sequencing

A multigene panel test was performed, including 18 genes associated with RASopathies: *PTPN11, RAF1, RIT1, KRAS, NRAS, BRAF, LZTR1, CBL, HRAS, MAP2K1, MAP2K2, NF1, RASA1, SHOC2, SOS1, SOS2, SPRED1,* and *PPP1CB*. Peripheral vein blood samples were collected from patients, and genomic DNA was isolated using the QIAamp® DNA Blood Mini Kit with the QIAcube® system (Qiagen, Germany). The isolated DNA then underwent library preparation following the manufacturer's protocol. Quantification of the prepared library was performed using Real-Time PCR (Rotor-Gene) with the QIAseq Library Quant Assay Kit. Data analysis was conducted using raw data from the prepared library, which was loaded into the MiSeq system. The data were then filtered in Variant Call Format to identify causative variants.

Confirmation by Sanger Sequencing

Sanger sequencing was conducted to confirm the reported variants and for segregation. Specific primers were designed for the region of interest, which was subsequently amplified using the PCR technique. Sequencing was performed using the Applied Biosystems AB3500 Genetic Analyzer. The results were compared with the reference gene sequence from the NCBI GenBank database and analyzed utilizing the Mutation Surveyor program. Patients who underwent Sanger sequencing are listed in Table 3.

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Table 1. Findings of cases diagnosed with NF1 and NF1-NS

Patient ID	Sex	Age	Mother's age at birth	Father's age at birth	NIH diagnostic criteria							Additional findings							
					CALM (≥6)	freckling	neurofibroma	Lisch nodule	optic glioma	skeletal findings	family history	cardiac	scoliosis	neuroimaging	psychiatric findings/ behavioral abnormalities	headache/ dizziness	seizures	abdominal ultrasound findings	other
1	F	34 y	N/A	N/A	+	+	+	+	−	−	+	+	−	−	−	−	−	−	
2	M	15 y 1 m	24	36	+	N/A	−	+	−	−	−	−	−	Bilateral deep gray matter lesions, thickened corpus callosum	+	−	−	−	
3	M	11 y 7 m	21	31	+	+	−	−	−	−	+	−	−	T2-FLAIR hyperintense and diffusion-restricted foci in dentate nuclei, brainstem, and basal ganglia	−	+	−	−	
4	M	10 y 7 m	41	55	+	+	−	+	−	−	−	−	+	T2-FLAIR hyperintense hamartomatous lesions with diffusion restriction in thalamus, internal capsule, basal ganglia, hippocampi, and dentate nuclei	+	−	−	−	
5	M	29 y	31	36	+	+	+	+	−	−	+	+	+	3 nodular lesions with fibrotic margins in the left frontal scalp; right frontal subcortical T2-FLAIR hyperintense focus; plexiform neurofibromas and preorbital thickening on orbital MRI	−	−	−	−	Syndactyly bilaterally at feet
6	M	3 y 4 m	34	36	+	+	−	−	−	−	−	+	+	Multiple diffusion-restricted hamartomatous lesions in deep gray matter and brainstem	+	−	−	−	Relative macrocephaly
7	F	39 y	21	N/A	+	+	+	N/A	N/A	N/A	+	N/A	N/A	FLAIR hyperintense hamartoma-like lesion extending from left thalamus to temporal lobe; neurofibroma-like lesions on cervical skin	N/A	+	−	−	Pectus excavatum
8	M	15 y 1 m	27	28	+	+	N/A	+	−	−	+	N/A	−	T2/FLAIR hyperintense hamartomatous lesions in bilateral globus pallidus, thalami, hippocampi, and dentate nuclei with thickened corpus callosum; arachnoid cyst in the retrocerebellar area	+	−	−	Accessory spleen	

Table 1 (continued)

Patient ID	Sex	Age	Mother's age at birth	Father's age at birth	NIH diagnostic criteria							Additional findings							
					CALM (≥6)	freckling	neurofibroma	Lisch nodule	optic glioma	skeletal findings	family history	cardiac	scoliosis	neuroimaging	psychiatric findings/ behavioral abnormalities	headache/ dizziness	seizures	abdominal ultrasound findings	other
9	F	18 y 1 m	22	36	+	+	+	N/A	N/A	–	+	N/A	–	–	–	+	–	–	
10	F	9 y 11 m	31	N/A	+	–	+	+	N/A	N/A	+	+	+	Brainstem and deep gray matter hamartomas with thickened corpus callosum; neurofibroma at T12–L1	+	+	–	Duplex collecting system	
11	M	3 y 2 m	23	24	+	+	N/A	+	–	–	+	+	+	T2/FLAIR hyperintense non-enhancing foci in right cerebral and bilateral cerebellar deep white matter; thoracic hydrosyringomyelia; mild optic nerve tortuosity and thickening bilaterally	+	–	+	–	
12	F	5 y	20	25	+	N/A	N/A	N/A	N/A	–	+	N/A	–	N/A	–	N/A	–	N/A	
13[a]	M	8 m	50	45	+	N/A	N/A	N/A	N/A	N/A	–	N/A	–	Increased T2/FLAIR signal in bilateral parieto-occipital area; diffusion restriction right parieto-occipital area; increased diffusion of posterior right thalamus	+	–	+	Bilaterally prominent pelvicalyceal system	Pectus carinatum, Noonan-like facial dysmorphism
14	M	12 y 1 m	19	27	+	+	–	N/A	N/A	–	–	–	+	T2/FLAIR hyperintensities with increased diffusion in dentate nuclei, right brachium pontis, thalami, cerebral peduncles, bilateral hippocampi, left globus pallidus	–	–	–	N/A	Wing scapula
15	M	10 y 4 m	33	36	+	+	+	N/A	N/A	–	+	N/A	–	T2/FLAIR hyperintense lesions with diffusion increase in bilateral globus pallidus, thalami, dentate nuclei, hippocampi, anterior tegmentum, and tectum (adjacent to 4th ventricle)	–	–	–	–	Relative macrocephaly

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Table 1 (continued)

Patient ID	Sex	Age	Mother's age at birth	Father's age at birth	NIH diagnostic criteria							Additional findings							
					CALM (≥6)	freckling	neurofibroma	Lisch nodule	optic glioma	skeletal findings	family history	cardiac	scoliosis	neuroimaging	psychiatric findings/ behavioral abnormalities	headache/ dizziness	seizures	abdominal ultrasound findings	other
16	F	14 y 4 m	22	30	+	+	+	+	–	+	+	–	–	Multiple hamartomatous lesions in bilateral dentate nuclei, brachium pontis, cerebral peduncles, posterior thalami, and globus pallidus, plexiform neurofibroma adjacent to right periorbital fat and sphenoid bone, temporal arachnoid cyst, numerous neurofibromas in thoracic, lumbar, and sacral vertebrae	–	+	–	–	Ptosis, camptodactyly, sphenoid wing dysplasia
17	F	12 y 4 m	32	37	+	+	N/A	+	–	N/A	–	N/A	N/A	Increased diffusion in bilateral globus pallidus	–	+	–	–	Relative macrocephaly, ptosis
18	M	42 y	N/A	N/A	+	N/A	+	+	–	–	+	N/A	+	Central atrophy, orbital and parieto-occipital neurofibromas, craniocervical myelopathy, extensive spinal and soft tissue neurofibromas	–	–	–	–	Decrease in muscle strength, multiple nerve sheath tumor
19	M	32 y	N/A	N/A	+	N/A	+	N/A	N/A	N/A	+	N/A	N/A	N/A	N/A	–	–	N/A	
20	F	2 y 6 m	26	37	+	+	+	–	–	–	+	–	+	T2/FLAIR hyperintense lesions with diffusion restriction in deep gray matter and brainstem structures, optic chiasm hypertrophy	–	–	–	–	
21	F	24 y	N/A	N/A	+	+	+	+	N/A	N/A	+	N/A	N/A	N/A	–	+	–	N/A	
22	F	8 y 10 m	25	33	+	N/A	N/A	–	–	N/A	–	–	N/A	Hamartomatous lesion with diffusion restriction in left cerebellar peduncle	–	+	–	–	Macrocephaly, coarse face
23	M	8 y 9 m	31	37	+	N/A	–	–	–	–	–	–	–	T2/FLAIR hyperintense foci with increased diffusion in deep gray matter and hypothalamus	+	–	–	–	Relative macrocephaly, pectus excavatum

Table 1 (continued)

Patient ID	Sex	Age	Mother's age at birth	Father's age at birth	NIH diagnostic criteria							Additional findings							
					CALM (≥6)	freckling	neurofibroma	Lisch nodule	optic glioma	skeletal findings	family history	cardiac	scoliosis	neuroimaging	psychiatric findings/ behavioral abnormalities	headache/ dizziness	seizures	abdominal ultrasound findings	other
24	F	31 y	N/A	N/A	+	+	+	N/A	–	–	+	+	–	Focal FLAIR hyperintensity near right lateral ventricle body	–	–	–	–	Multiple sclerosis
25	F	5 y	30	28	+	+	–	–	+	–	–	–	+	Left temporal arachnoid cyst, multifocal brainstem, deep gray matter hyperintensities, and right optic chiasm glioma	–	–	–	–	Inward rotation of the feet
26	F	40 y	28	N/A	+	+	+	+	–	–	+	N/A	+	–	–	+	–	–	
27	F	9 y 7 m	32	29	+	–	–	–	–	–	+	+	+	Focal hyperintense focus in the posterior limb of the left internal capsule	–	–	–	–	Strabismus
28	M	4 y 3 m	43	40	+	N/A	N/A	N/A	N/A	N/A	–	N/A	N/A	N/A	–	–	–	–	Relative macrocephaly
29	F	17 y	N/A	N/A	+	–	+	+	–	N/A	+	N/A	N/A	N/A	–	–	–	N/A	
30	M	25 y	N/A	N/A	+	N/A	+	–	–	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
31	M	9 y	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	+	N/A	N/A	Focal T2/FLAIR hyperintensities in the deep gray matter of bilateral globus pallidus and left cerebellar hemisphere on supratentorial slices	N/A	N/A	N/A	N/A	Relative macrocephaly
32	F	29 y	N/A	N/A	+	+	+	N/A	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
33	M	21 y	N/A	N/A	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
34	M	47 y	N/A	N/A	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
35	F	17 y	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Recurrent oral aphthous
36	F	7 m 3 d	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
37	M	24 y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
38	M	13 y	31	N/A	+	+	N/A	N/A	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Mass in the nipple
39	F	3 y	N/A	N/A	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
40	M	7 y	N/A	N/A	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	T2 hyperintense lesions in globus pallidus interna and right midbrain	+	N/A	N/A	N/A	Short stature
41	M	9 y	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Right cerebellar hamartoma	+	N/A	N/A	N/A	

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Table 1 (continued)

Patient ID	Sex	Age	Mother's age at birth	Father's age at birth	NIH diagnostic criteria							Additional findings							
					CALM (≥6)	freckling	neurofibroma	Lisch nodule	optic glioma	skeletal findings	family history	cardiac	scoliosis	neuroimaging	psychiatric findings/ behavioral abnormalities	headache/ dizziness	seizures	abdominal ultrasound findings	other
42	F	40 y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	+	N/A	N/A	Intracranial schwannoma
43	F	17 y 3 m	33	33	+	+	–	+	–	N/A	+	–	N/A	T2/FLAIR hyperintense foci with increased diffusion in bilateral anterior hypothalamus and thalamus	+	–	–	–	Relative macrocephaly
44	F	55 y	N/A	N/A	+	+	+	+	–	–	+	–	N/A	N/A	–	–	–	N/A	
45[a]	M	16 y 9 m	N/A	N/A	+	+	+	N/A	N/A	+	+	N/A	–	N/A	+	–	–	N/A	Short stature, strabismus
46	F	38 y	N/A	N/A	+	–	+	–	–	–	+	N/A	–	T2/FLAIR hyperintense foci in the deep gray matter	–	+	–	N/A	
47	F	40 y	N/A	N/A	+	+	+	–	–	–	+	+	–	T2/FLAIR hyperintense foci in the deep white matter and basal ganglia regions	–	–	–	–	
48	M	34 y	N/A	N/A	+	+	–	N/A	N/A	–	+	N/A	–	T2/FLAIR hyperintense foci in the left periventricular white matter at the supratentorial level, bilateral cerebellar folia prominence with sequelae changes	–	–	–	–	MVP, strabismus, splenomegaly
49	F	31 y	N/A	N/A	+	+	+	N/A	N/A	–	+	N/A	–	–	–	+	–	N/A	Macrocephaly
50	F	16 y 4 m	N/A	N/A	+	+	–	–	–	–	+	N/A	–	N/A	–	–	–	N/A	
51	M	5 y 4 m	29	50	+	–	–	–	–	+	–	–	+	N/A	+	–	–	Fused and ectopically located kidneys consistent with horseshoe and ectopic kidney anomaly	IVF pregnancy, hypospadias, speech delay, anal atresia, pectus excavatum, relative macrocephaly
Total					49/49	31/36	21/33	15/27	1/26	3/28	32/43	8/20	12/29	28/32	13/38	12/38	2/38	4/28	

CALM, café-au lait macule; MVP, mitral valve prolapse; MRI, magnetic resonance imaging; N/A, not available. [a]Patients 13 and 45 are diagnosed with neurofibromatosis-NS and others with NF1 syndrome.

Multiplex Ligation-Dependent Probe Amplification

To detect deletions and duplications associated with NF1, patient 43 – who showed no abnormalities on sequence analysis – was evaluated using Multiplex Ligation-Dependent Probe Amplification (MLPA) analysis targeting the *NF1* gene. MLPA was performed with MRC Holland Salsa MLPA Probe Mix P081-D1 and P082-C2 region kits, and the results were analyzed using the Coffalyser program.

Variant Analysis and Interpretation

The pathogenicity of variants was evaluated using the Qiagen Clinical Insight (QCI) Interpret software, following the classification guidelines established by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) [6, 7]. Additionally, results from ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), ClinGen RASopathy Variant Curation Expert Panel (VCEP) guidelines, Franklin (https://franklin.genoox.com/clinical-db/home), and VarSome (https://varsome.com) were utilized in annotation and classification [8–10]. Several in silico prediction tools, including CADD (https://cadd.gs.washington.edu), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), SIFT (https://sift.bii.a-star.edu.sg), MutationTaster (https://www.mutationtaster.org), and QCI Inferred Activation, were used to assess the effects of the variants. Variants classified through these tools were further evaluated in combination with family segregation analyses, and variants considered clinically significant and potentially disease-causing were reported. After completing all the steps, this study evaluated variants classified as pathogenic, likely pathogenic, and variant of uncertain significance (VUS) that were considered potential causes of disease after clinical correlation.

Results

The study included 76 cases who presented to our outpatient clinic over a 6-year period and were diagnosed with RASopathy based on both clinical and molecular findings. Including family members who were also molecularly analyzed, the total number of cases in the study was 98.

The diagnostic yield based on NF1 clinical diagnostic criteria is presented in Table 1. Clinical manifestations and diagnosis of patients carrying pathogenic variants in genes other than *NF1* are detailed in Table 2.

Of the 76 index cases, 44.7% (*n*: 34) were female, 52.6% (*n*: 40) were male, and 2.6% (*n*: 2) were antenatal cases with unknown genders. The average age of female cases was 18.4 years, while that of male cases was 14.4 years. The overall age range extended from 11 + 6/7 gestational weeks in an antenatal case (case 75, diagnosed with a *RAF1* variant) to 55 years old (patient 44, diagnosed with NF1). Among patients with variants in the *NF1* gene, the average age was 18.9 years, compared to 10.2 years for those with variants in other genes. For cases with variants detected in the *PTPN11* gene, the average age was 10.1 years.

Of the 40 patients who underwent segregation analysis, 18 (45%) harbored de novo variants. Among the remaining cases, 14 (35%) inherited the variant paternally and 8 (20%) maternally. Within the NF1 subgroup, 8 cases (29.6%) were identified as de novo. For familial NF1 cases, the variant origin was paternal in 63.6% and maternal in 36.4% of cases (Table 3). The distribution of variants according to gene and classification is shown in Figure 1.

The majority of variants detected in the *NF1* gene (12%) were located in exon 21, while most variants in the *PTPN11* gene (36%) were found in exon 3. A total of 75% of variants detected in the *RAF1* gene were located in exon 7, with 2 cases sharing the same variant. Variants in the *SOS1* gene were found in different locations across the gene.

Among the alterations in the *NF1* gene, 33% (*n*: 17) were frameshift, 29% (*n*: 15) were nonsense, and 21.5% (*n*: 11) were missense variants. In contrast, among the non-*NF1* RASopathy cases, 96% (*n*: 24) of the identified alterations were missense variants.

A total of 14 novel variants were identified, including one in the *SOS1* gene and the remaining in the *NF1* gene (Table 3). Of the diagnosed cases, 49 were diagnosed with NF1, 2 with neurofibromatosis-NS, 21 with NS, 2 with NSML, and 1 with cardiofaciocutaneous syndrome. Additionally, a pathogenic variant in the *RAF1* gene was identified in an antenatal case (patient 75). A postnatal examination was recommended to confirm the diagnosis of NS or NSML. The most common diagnosis was NF1, followed by NS, making these two syndromes the majority within the study group.

Discussion

RASopathies are among the most common genetic syndrome groups, with NS being the most prevalent type, occurring in approximately 1 in 1,000 to 2,500 live

Table 2. Clinical manifestations of patients with pathogenic variants in genes other than *NF1*

Patient ID	Sex	Age	Mother's age at birth	Father's age at birth	Cardiac	Short stature	Skeletal findings	Facial dysmorphism	Other	Diagnosis
52	M	10 y 11 m	29	34	+	+	+	+	Hypothyroidism, cryptorchidism	Noonan
53	M	26 y	21	23	–	+	+	+	–	Noonan
54	M	12 y 2 m	33	34	+	+	–	+	Antenatal polyhydramnios, autism spectrum disorder	Noonan
55	F	12 y 10 m	27	29	+	+	+	+	Failure to thrive, CALM, nasal speech, left renal agenesis	NSML
56	M	24 y	36	38	+	+	+	+	Psychomotor retardation, cryptorchidism, strabismus, curly hair, laterally sparse eyebrows, truncal obesity	CFCS
57	M	1 m 11 d	31	32	+	+	+	+	Antenatal polyhydramnios and hydronephrosis, failure to thrive, enlargement of the lateral and third ventricles, neuromotor retardation	Noonan
58	M	9 y 1 m	30	36	N/A	+	+	+	Failure to thrive, psychomotor retardation, hydrocephalus, splenomegaly, a solid-cystic formation in the pelvis	Noonan
59	M	1 m 26 d	28	27	+	+	–	+	Cryptorchidism, hepatosplenomegaly, anemia, thrombocytopenia	Noonan
60	M	18 y 8 m	N/A	N/A	+	+	+	+	Glaucoma, myopia, splenomegaly, microcephaly, failure to thrive	Noonan
61	F	4 y	37	37	+	+	+	+	Neonatal hypotonia, developmental delay, nephrocalcinosis, wooly hair, pseudoexotropia	Noonan
62	M	5 y 4 m	25	25	+	+	+	+	Antenatal polyhydramnios, neonatal hypotonia, cryptorchidism, ptosis, amblyopia, myopia, renal cyst	Noonan
63	F	7 m 11 d	26	24	+	+	+	+	IUGR, microcephaly, failure to thrive	Noonan
64	M	1 y 25 d	28	33	–	–	–	+	JMML, transformation from JMML to AML resulting in death at 18 months of age, leukocytosis, thrombocytopenia, neutropenia, recurrent infection, strabismus	Noonan
65	M	11 y 2 m	40	33	–	+	+	+	Growth retardation, microcephaly, intellectual disability, glaucoma, a history of occasional febrile convulsions	Noonan
66	M	6 y 1 m	27	28	–	+	+	+	Prenatal and postnatal growth retardation, strabismus, amblyopia, myopia	Noonan
67	F	13 y 7 m	31	32	+	+	+	+	Multiple lentigines, growth retardation, LVH, HCM, strabismus	NSML
68	M	31 y	17	25	–	+	+	+	Feeding difficulties, strabismus, myopia, constipation	Noonan

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Table 2 (continued)

Patient ID	Sex	Age	Mother's age at birth	Father's age at birth	Cardiac	Short stature	Skeletal findings	Facial dysmorphism	Other	Diagnosis
69	M	12 y 1 m	25	33	+	+	+	+	Growth retardation, microcephaly, strabismus, optic atrophy	Noonan
70	F	6 y 4 m	N/A	N/A	+	+	+	+	Growth retardation, developmental delay, articulation disorder, microcephaly, hypodontia	Noonan
71	M	4 m	N/A	N/A	+	+	+	+	Hypotonia, failure to thrive, microcephaly, hypospadias, cryptorchidism	Noonan
72	F	19 y	N/A	N/A	+	–	+	+	HCM, microcephaly	Noonan
73	F	4 y	N/A	N/A	+	N/A	N/A	N/A	–	Noonan
74	Antenatally 24 w + 2/7 d		39	N/A	+	–	–	–	Antenatally hydrops fetalis, single umbilical artery, TR, polyhydramnios, increased nuchal translucency	Noonan
75	Antenatally 11 w + 6/7 d		31	N/A	–	–	–	–	IVF pregnancy, antenatally cystic hygroma	Noonan/ NSML
76	F	8 y 3 m	27	29	+	–	–	+	Developmental delay, intellectual disability, ADHD, aggressive behavior, hydronephrosis, urinary incontinence, curly hair, delayed hair growth	Noonan
Total					18/24	19/24	18/24	22/24		

N/A, not available; CALM, café-au-lait macule; NSML, Noonan syndrome with multiple lentigines; CFCS, cardiofaciocutaneous syndrome; IUGR, intrauterine growth restriction; JMML, juvenile myelomonocytic leukemia; AML, acute myelogenous leukemia; HCM, hypertrophic cardiomyopathy; LVH, left ventricular hypertrophy; TR, tricuspid regurgitation; IVF, in vitro fertilization; ADHD, attention-deficit and hyperactivity disorder; y, year; m, month; w, week; d, day.

births. The second most common RASopathy is NF1, with a frequency of about 1 in 3,000 births [5, 11, 12]. In our study, these two syndromes were the most frequently detected, consistent with the literature. NF1 was the most prevalent syndrome within the RASopathy group. One possible explanation for the lower prevalence of NS in our findings, compared to the literature, is that individuals without significant cardiac or other systemic anomalies may often remain undiagnosed. Alternatively, although severe presentations of NS are relatively rare, such cases are typically recognized earlier due to the prominence of clinical features prompting genetic evaluation. Additionally, NF1 is generally more familiar to healthcare professionals than other RASopathies, which may contribute to its relatively higher diagnostic rate.

The average age of diagnosis for NS has been reported as 9 years [13], and our findings are consistent with this observation. In contrast, the age at diagnosis for NF1 in our study was higher than that for other RASopathy syndromes. This may be attributed to the fact that, although NF1 is classified as a RASopathy, its clinical presentation differs markedly from other syndromes in this group. Moreover, NF1 is frequently underdiagnosed in early childhood due to its highly variable presentation and progressive nature. Many of its characteristic ectodermal manifestations – such as café-au-lait macules (CALMs) and neurofibromas – tend to emerge or become more pronounced during adolescence, which can delay clinical recognition and diagnosis.

In the literature, the de novo variant rate for NF1 cases is reported to be 42–50% [14, 15]. Approximately 50% of NS cases are sporadic [16]. In our study, 29.6% of NF1 variants were de novo. Among non-NF1 cases with available information – including 1 de novo case of NSML and the remaining cases of NS – 10 out of 12 (83.3%) had de novo variants, while in 2 cases (16.6%), the variant was inherited from the father (Table 3). Generally, 45% of variants detected as de novo. Increasing paternal age is associated with a higher risk of de novo variants favoring transmission to males [17], while maternal age does not appear to influence this risk [18, 19]. In 71% of de novo NF1 cases, the paternal age was over 35 years, whereas in non-NF1 de novo cases, paternal age exceeded 35 years in only 22%. De novo variants can occur even within the same family [20]. Recognizing the possibility of independent de novo variants occurring within the same family is also important for genetic counseling and prenatal testing in affected families.

A comparison of NF1 finding frequencies with the literature is presented in Table 4. Our study group aligns with the literature in terms of the frequency of CALMs, intertriginous freckling, neurofibromas, cranial magnetic resonance imaging findings, seizure history, and malignant peripheral nerve sheath tumors. However, the rates of Lisch nodules, optic gliomas, and learning disabilities are lower than expected. This discrepancy may be attributed to the younger age of many cases in our study group, as some conditions may manifest later in life. Plans are in place to conduct long-term follow-ups on these cases as they age. Furthermore, the limited number of cases and the tendency of patients to neglect necessary medical follow-ups may have contributed to delays in detecting certain findings. In contrast, the rates of scoliosis, and cardiac findings were notably higher compared to the literature. The higher rates observed in our study may be attributed to the small number of patients.

In the study by Nguyen et al. [21], when comparing the rate of cardiac findings between individuals with NF1 large gene deletions and those without deletions, no cardiac abnormalities were observed in non-deletion NF1 cases, whereas cardiac findings were detected in 10 out of 16 cases with the deletion. This association is likely due to haploinsufficiency of one or more genes within the deleted region that are involved in cardiac development. Notably, *ADAP2*, *SUZ12*, and *UTP6* – genes affected by the deletion – are highly expressed during early heart development in both mice and human fetuses [22]. Nevertheless, it is important to note that cardiac findings may still be present in NF1 as part of the broader RASopathy phenotype, even in the absence of features overlapping with NS. In our study, patient 43 had NF1 whole-gene deletion (Fig. 2); however, she exhibited no cardiac defects. Incecik et al. [23] reported that cardiac abnormalities were present in 11 out of 65 patients (15.3%) in Turkish population. However, these rates need further validation through similar studies conducted in larger cohort groups within Turkish population.

NF1 deletions are associated with more severe clinical manifestations, including a higher risk of malignancy, cardiac abnormalities, intellectual disability, dysmorphic facial features, and overgrowth [24]. Loss-of-function variants in *SUZ12*, a gene frequently co-deleted with *NF1*, have also been linked to overgrowth, suggesting that neighboring genes within the deleted region may contribute to the broader phenotype observed in some *NF1* deletion cases. Patient 43, who had a whole-gene *NF1* deletion, exhibited intellectual disability,

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Table 3. Molecular characteristics of variants detected in patients

Patient ID	Gene	Transcript	Variant	Peptide	Mutation type	ACMG[a]	dbSNP (rs number)	Database	Location	Publication (PMID)	Sanger sequencing for confirmation	Sanger sequencing for segregation analysis
1	*NF1*	NM_001042492.3	c.3529dup	p.(Ala1177GlyfsTer18)	frameshift	LP (PVS1, PM2)	–	ClinVar: –	Exon 27	Novel	+	N/A
2	*NF1*	NM_001042492.3	c.4725–2A>G	–	Intronic, splice site	P (PVS1, PS4, PM2)	rs1295045178	ClinVar (VCV001073883.5): P/LP	Intron 35	31533651, 18546366	+	De novo
3	*NF1*	NM_001042492.3	c.4084C>T	p.(Arg1362Ter)	Nonsense	P (PVS1, PS4, PM2)	rs137854560	ClinVar (VCV000000344.38): P	Exon 30	10543400, 12112660	+	+ (father)
4	*NF1*	NM_001042492.3	c.2352G>C	p.(Trp784Cys)	Missense	P (PS1, PS4, PM2, PM5, PP2)	rs199474778	ClinVar (VCV000068319.8): P/LP	Exon 20	35885913, 10980545	N/A	De novo
5	*NF1*	NM_001042492.3	c.2498_2501del	p.(Ser833TyrfsTer7)	Frameshift	LP (PVS1, PM2)	–	ClinVar: –	Exon 21	Novel	+	+ (father)
6	*NF1*	NM_001042492.3	c.795del	p.(Val266PhefsTer15)	Frameshift	P (PVS1, PM2, PM6)	–	ClinVar: –	Exon 8	Novel	+	De novo
7	*NF1*	NM_001042492.3	c.2850G>T	p.(Gln950His)	Missense, splice site	LP (PS1, PM2, PP2)	rs863224446	ClinVar (VCV002763795.2): VUS	Exon 21	31717729, 26740943	+	+ (father)
8	*NF1*	NM_001042492.3	c.2850G>T	p.(Gln950His)	Missense, splice site	LP (PS1, PM2, PP2)	rs863224446	ClinVar (VCV002763795.2): VUS	Exon 21	31717729, 26740943	+	+ (mother)
9	*NF1*	NM_001042492.3	c.4522C>G	p.(His1508Asp)	Missense	VUS (PM2, PP3, PP2)	rs1393895345	ClinVar (VCV000996411.3): VUS	Exon 34	–	+	+ (father)
10	*NF1*	NM_001042492.3	c.1885G>A	p.(Gly629Arg)	Missense	LP (PS4, PM2, PS3, PP2)	rs199474738	ClinVar (VCV000068308.56): P	Exon 17	26056819, 29914388	+	+ (father)
11	*NF1*	NM_001042492.3	c.6950G>A	p.(Trp2317Ter)	Nonsense	LP (PVS1, PM2)	rs2069790543	ClinVar (VCV000848870.5): P	Exon 47	–	+	+ (father)
12	*NF1*	NM_001042492.3	c.4577+2dup	–	Intronic, splice site	LP (PM2, PP1, PP3, PP5)	rs2067666266	ClinVar (VCV000846370.9): conflicting (P/VUS)	Intron 34	–	+	+ (mother)

Table 3 (continued)

Patient ID	Gene	Transcript	Variant	Peptide	Mutation type	ACMG[a]	dbSNP (rs number)	Database	Location	Publication (PMID)	Sanger sequencing for confirmation	Sanger sequencing for segregation analysis
13	*NF1*	NM_001042492.3	c.2540T>C	p.(Leu847Pro)	Missense	P (PP1, PM2, PM5, PS3, PP2, PP3)	rs199474747	ClinVar (VCV000068323.55): P/LP	Exon 21	29290338, 23668869	N/A	N/A
14	*NF1*	NM_001042492.3	c.248A>C	p.(Gln83Pro)	Missense	P (PS4, PM1, PM2, PP1, PP2)	rs1060500360	ClinVar (VCV000641956.8): P/LP	Exon 3	23913538	N/A	N/A
15	*NF1*	NM_001042492.3	c.7909C>T	p.(Arg2637Ter)	Nonsense	P (PVS1, PS4, PM2, PM6)	rs786201367	ClinVar (VCV000184261.79): P/LP	Exon 54	30530636, 10543400	N/A	De novo
16	*NF1*	NM_001042492.3	c.5570del	p.(Ile1857ThrfsTer6)	Frameshift	LP (PVS1, PM2)	–	ClinVar: –	Exon 38	Novel	N/A	+ (mother)
17	*NF1*	NM_001042492.3	c.5768C>G	p.(Thr1923Arg)	Missense	LP (PM2, PM5, PP3, PP2)	rs786203824	ClinVar (VCV000404539.10): LP	Exon 39	25074460	N/A	N/A
18	*NF1*	NM_001042492.3	c.2110_2111dup	p.(Val705TrpfsTer44)	Frameshift	LP (PVS1, PM2)	–	ClinVar: –	Exon 18	Novel	+	+ (mother)
19	*NF1*	NM_001042492.3	c.5552dup	p.(Gly1852TrpfsTer10)	Frameshift	LP (PVS1, PM2)	–	ClinVar: –	Exon 38	18546366	N/A	+ (mother)
20	*NF1*	NM_001042492.3	c.3739_3742del	p.(Phe1247IlefsTer18)	Frameshift	P (PVS1, PS4, PM2)	rs1064794276	ClinVar (VCV000420078.82): P	Exon 28	18546366, 12807981	+	+ (mother)
21	*NF1*	NM_001042492.3	c.7468G>T	p.(Glu2490Ter)	Nonsense	LP (PVS1, PM2)	rs2151576916	ClinVar: –	Exon 51	–	N/A	N/A
22	*NF1*	NM_001042492.3	c.4217_4220del	p.(Gly1406ValfsTer21)	Frameshift	P (PVS1, PM2, PM6)	–	ClinVar: –	Exon 32	Novel	+	De novo
23	*NF1*	NM_001042492.3	c.2542G>C	p.(Gly848Arg)	Missense	P (PP1, PS1, PP2, PM2, PM5, PM6, PP3)	rs1060500368	ClinVar (VCV000404588.15): P/LP	Exon 21	29290338, 27171602	+	De novo

Table 3 (continued)

Patient ID	Gene	Transcript	Variant	Peptide	Mutation type	ACMG[a]	dbSNP (rs number)	Database	Location	Publication (PMID)	Sanger sequencing for confirmation	Sanger sequencing for segregation analysis
24	*NF1*	NM_001042492.3	c.5902C>T	p.(Arg1968Ter)	Nonsense	P (PVS1, PS4, PM2)	rs137854552	ClinVar (VCV000000343.52): P	Exon 40	33443663, 10076878	N/A	N/A
25	*NF1*	NM_001042492.3	c.3152del	p.(Gly1051GlufsTer11)	Frameshift	P (PVS1, PM2, PM6)	rs2151432442	ClinVar (VCV001685975.2): P	Exon 24	–	+	De novo
26	*NF1*	NM_001042492.3	c.574C>T	p.(Arg192Ter)	Nonsense	P (PVS1, PS4, PM2)	rs397514641	ClinVar (VCV000040093.110): P	Exon 5	16835897, 27838393	N/A	+ (father)
27	*NF1*	NM_001042492.3	c.5610−−2A>G	–	Intronic, splice site	P (PVS1, PS4, PM2)	rs1135402876	ClinVar (VCV000457756.10): P	Intron 38	26969325, 25074460	+	+ (father)
28	*NF1*	NM_001042492.3	c.1541_1542del	p.(Gln514ArgfsTer43)	Frameshift	P (PVS1, PS4, PM2)	rs267606600	ClinVar (VCV000000346.90): P	Exon 14	24676424, 26969325	N/A	N/A
29	*NF1*	NM_001042492.3	c.7062+2T>G	–	Intronic, splice site	LP (PVS1, PM2)	–	ClinVar:–	Intron 47	Novel	+	N/A
30	*NF1*	NM_001042492.3	c.2071_2074dup	p.(Tyr692SerfsTer9)	Frameshift	P (PVS1, PM2)	–	ClinVar: –	Exon 18	Novel	+	N/A
31	*NF1*	NM_001042492.3	c.2409+2dup	–	Intronic, splice site	VUS (PM2, PP1)	–	ClinVar (VCV003722626.1): VUS	Intron 20	18546366	+	+ (father)
32	*NF1*	NM_001042492.3	c.3132C>A	p.(Tyr1044Ter)	Nonsense	P (PVS1, PS4, PM2)	rs779047683	ClinVar (VCV001366138.7): P	Exon 24	25325900	N/A	N/A
33	*NF1*	NM_001042492.3	c.7439_7451del	p.(His2480ProfsTer5)	Frameshift, splice site	LP (PVS1, PM2)	–	ClinVar: –	Exon 50	Novel	+	N/A
34	*NF1*	NM_001042492.3	c.2329T>A	p.(Trp777Arg)	Missense, splice site	P (PS1, PM2, PM5, PP1, PP2, PP3)	rs876658853	ClinVar (VCV000230937.13): P/LP	Exon 20	17726231, 23656349	N/A	N/A
35	*NF1*	NM_001042492.3	c.2503C>T	p.(Gln835Ter)	Nonsense	P (PVS1, PS4, PM2)	rs1555614207	ClinVar (VCV000523904.12): P/LP	Exon 21	29617658	N/A	N/A
36	*NF1*	NM_001042492.3	c.2880dup	p.(Val961CysfsTer14)	Frameshift	LP (PVS1, PM2)	–	ClinVar: –	Exon 22	Novel	+	+ (father)

Table 3 (continued)

Patient ID	Gene	Transcript	Variant	Peptide	Mutation type	ACMG[a]	dbSNP (rs number)	Database	Location	Publication (PMID)	Sanger sequencing for confirmation	Sanger sequencing for segregation analysis
37	*NF1*	NM_001042492.3	c.5352T>A	p.(Tyr1784Ter)	Nonsense	LP (PVS1, PM2)	–	ClinVar (VCV002754327.2): P	Exon 38	18041031	N/A	N/A
38	*NF1*	NM_001042492.3	c.4783C>T	p.(Gln1595Ter)	Nonsense	P (PVS1, PS4, PM2)	rs1597753263	ClinVar (VCV000649568.8): P	Exon 36	29685074, 23460398	N/A	+ (mother)
39	*NF1*	NM_001042492.3	c.6852_6855del	p.(Tyr2285ThrfsTer5)	Frameshift	P (PVS1, PM2, PM6)	rs1555535032	ClinVar (VCV000216866.36): P	Exon 46	34988040, 34427956	N/A	De novo
40	*NF1*	NM_001042492.3	c.4600C>T	p.(Arg1534Ter)	Nonsense	P (PVS1, PS4, PM2)	rs760703505	ClinVar (VCV000220152.91): P/LP	Exon 35	31783133, 23668869	N/A	N/A
41	*NF1*	NM_001042492.3	c.7570del	p.(Leu2524TrpfsTer24)	Frameshift	LP (PVS1, PM2)	–	ClinVar: –	Exon 51	Novel	+	N/A
42	*NF1*	NM_001042492.3	c.7549C>T	p.(Arg2517Ter)	Nonsense	P (PVS1, PS4, PM2)	rs866445127	ClinVar (VCV000230467.103): P	Exon 51	16835897, 7981679	N/A	N/A
43	*NF1*		NF1 whole-gene deletion							33951044		
44	*NF1*	NM_001042492.3	c.2033dup	p.(Ile679AspfsTer21)	Frameshift	P (PVS1, PS4)	rs587781807	ClinVar (VCV000141513.84): P	Exon 18	30308447	N/A	+ (mother)
45	*NF1*	NM_001042492.3	c.4847_4859delinsAT	p.(Gly1616AspfsTer2)	Frameshift	LP (PVS1, PM2)	–	ClinVar: –	Exon 37	Novel	+	+ (father)
46	*NF1*	NM_001042492.3	c.6427+3A>C	–	Intronic, splice site	VUS (PM2)	–	ClinVar: –	Intron 42	Novel	+	N/A
47	*NF1*	NM_001042492.3	c.6546C>G	p.(Tyr2182Ter)	Nonsense	P (PVS1, PS4, PM2)	rs876659768	ClinVar (VCV000803373.9): P	Exon 43	12807981	+	+ (father)
48	*NF1*	NM_001042492.3	c.1381C>T	p.(Arg461Ter)	Nonsense	P (PVS1, PS4, PM2, PM1)	rs878853865	ClinVar (VCV000237514.58): P	Exon 12	16944272	N/A	N/A
49	*NF1*	NM_001042492.3	c.6772C>T	p.(Arg2258Ter)	Nonsense	P (PVS1, PS4, PM2)	rs876658541	ClinVar (VCV000230389.61): P	Exon 45	26962827	N/A	N/A
50	*NF1*	NM_001042492.3	c.3494T>C	p.(Ile1165Thr)	Missense, splice site	P (PS4, PM2, PM5, PP2, PP3)	rs786204211	ClinVar (VCV000955671.9): conflicting (P/LP/VUS)	Exon 26	24789688	+	N/A

Table 3 (continued)

Patient ID	Gene	Transcript	Variant	Peptide	Mutation type	ACMG[a]	dbSNP (rs number)	Database	Location	Publication (PMID)	Sanger sequencing for confirmation	Sanger sequencing for segregation analysis
51	*NF1*	NM_001042492.3	c.288+5G>A	–	Intronic, splice site	LP (PS4, PM2, PP3)	rs1555605409	ClinVar (VCV000642764.11): P/LP	Intron 3	27999334, 29618358	N/A	N/A
52	*PTPN11*	NM_002834.5	c.922A>G	p.(Asn308Asp)	Missense	P (PS3, PP2, PP3, PM2)	rs28933386	ClinVar (VCV000013326.139): P	Exon 8	26817465	N/A	N/A
53	*RIT1*	NM_006912.6	c.151G>T	p.(Asp51Tyr)	Missense	VUS (PM2, PP2)	rs869025190	ClinVar (VCV000183402.9): VUS	Exon 3	27226556	+	N/A
54	*RIT1*	NM_006912.6	c.244T>A	p.(Phe82Ile)	Missense	P (PS4, PM5, PM6, PP2, PP3)	rs869025194	ClinVar (VCV000183406.11): P	Exon 5	23791108	+	De novo
55	*PTPN11*	NM_002834.5	c.1403C>T	p.(Thr468Met)	Missense	P (PS3, PS4, PM1, PP2, PP3)	rs121918457	ClinVar (VCV000013331.96): P	Exon 12	12058348	N/A	N/A
56	*BRAF*	NM_004333.6	c.1403T>G	p.(Phe468Cys)	Missense	LP (PM2, PM5, PP2, PP3)	rs397507473	ClinVar: –	Exon 11	29084544	N/A	N/A
57	*RAF1*	NM_002880.4	c.782C>T	p.Pro261Leu	Missense	LP (PM5, PS3, PP3)	rs397516828	ClinVar (VCV000120246.15): P/LP	Exon 7	32059087, 17603482	+	+ (father)
58	*KRAS*	NM_004985.5	c.40G>A	p.(Val14Ile)	Missense	P (PS3, PS4, PM1, PM2, PM6, PP2, PP3)	rs104894365	ClinVar (VCV000012589.70): P	Exon 2	16474405, 18958496	+	De novo
59	*PTPN11*	NM_002834.5	c.417G>C	p.(Glu139Asp)	Missense	P (PS3, PS4, PS1, PM5, PM6, PP2, PP3)	rs397507520	ClinVar (VCV000040513.88): P	Exon 4	18372317, 22315187	+	De novo
60	*PTPN11*	NM_002834.5	c.922A>G	p.(Asn308Asp)	Missense	P (PS3, PP2, PP3, PM2)	rs28933386	ClinVar (VCV000013326.139): P	Exon 8	26817465, 32164556	+	N/A

Table 3 (continued)

Patient ID	Gene	Transcript	Variant	Peptide	Mutation type	ACMG[a]	dbSNP (rs number)	Database	Location	Publication (PMID)	Sanger sequencing for confirmation	Sanger sequencing for segregation analysis
61	*PTPN11*	NM_002834.5	c.182A>G	p.(Asp61Gly)	Missense	P (PS3, PS4, PM2, PM5, PM6, PP2, PP3)	rs121918461	ClinVar (VCV000013330.70): P	Exon 3	19251646, 12161469	+	De novo
62	*SOS1*	NM_005633.4	c.2536G>A	p.(Glu846Lys)	Missense	LP (PS3, PM2, PM6)	rs397517159	ClinVar (VCV000040706.37): P	Exon 16	18651097, 17143282	+	De novo
63	*PTPN11*	NM_002834.5	c.844A>G	p.(Ile282Val)	Missense, splice site	P (PS4, PM1, PS3, PP2)	rs397507529	ClinVar (VCV000040525.87): P	Exon 7	18372317, 11704759	+	N/A
64	*NRAS*	NM_002524.5	c.34G>A	p.(Gly12Ser)	Missense	P (PS4, PM2, PM5)	rs121913250	ClinVar (VCV000177778.28): P	Exon 2	10598665	N/A	N/A
65	*PTPN11*	NM_002834.5	c.853T>C	p.(Phe285Leu)	Missense, splice site	P (PS1, PM1, PM2, PM6, PP2, PP3)	rs397507531	ClinVar (VCV000040528.53): P	Exon 7	11704759, 32164556	+	De novo
66	*SOS1*	NM_005633.4	c.1074+5G>T	–	Intronic, splice site	VUS (PM2, PP1, BP7)	–	ClinVar: –	Intron 8	Novel	+	+ (father)
67	*RAF1*	NM_002880.4	c.770C>T	p.(Ser257Leu)	Missense	P (PS3, PM1, PM2, PM6)	rs80338796	ClinVar (VCV000013957.105): P	Exon 7	22389993, 25706034	+	De novo
68	*PTPN11*	NM_002834.5	c.205G>C	p.(Glu69Gln)	Missense	LP (PS4, PM1, PM2, PM6, PP2, PP3)	rs397507511	ClinVar (CV000040498.61): P	Exon 3	26817465, 15889278	+	De novo
69	*PTPN11*	NM_002834.5	c.236A>G	p.(Gln79Arg)	Missense	P (PM1, PM6, PP1, PP2, PP3, PS3)	rs121918466	ClinVar (VCV000013340.57): P	Exon 3	29084544, 26817465	+	De novo

Table 3 (continued)

Patient ID	Gene	Transcript	Variant	Peptide	Mutation type	ACMG[a]	dbSNP (rs number)	Database	Location	Publication (PMID)	Sanger sequencing for confirmation	Sanger sequencing for segregation analysis
70	*PTPN11*	NM_002834.5	c.182A>G	p.(Asp61Gly)	Missense	P (PS3, PS4, PM2, PM5, PM6, PP2, PP3)	rs121918461	ClinVar (VCV000013330.70): P	Exon 3	20651068, 12161469	+	De novo
71	*SOS2*	NM_006939.4	c.2690A>G	p.(Lys897Arg)	Missense	VUS (PP1, BP4)	rs1246293566	ClinVar: –	Exon 17	–	+	N/A
72	*SOS1*	NM_005633.4	c.1654A>G	p.(Arg552Gly)	Missense	P (PS3, PM1, PM2, PM5_M, PP3)	rs137852814	ClinVar (VCV000012871.78): P	Exon 10	28957739, 31560489	N/A	N/A
73	*PTPN11*	NM_002834.5	c.417G>C	p.(Glu139Asp)	Missense	P (PS1, PS3, PS4, PM5, PP2, PP3)	rs397507520	ClinVar (VCV000040513.88): P	Exon 4	18372317, 22315187	N/A	N/A
74	*RIT1*	NM_006912.6	c.246T>G	p.(Phe82Leu)	Missense, splice site	P (PS1, PS3, PM1, PM2, PP2, PP3)	rs730881014	ClinVar (VCV000181522.69): P/LP	Exon 5	27699752, 23791108	N/A	N/A
75	*RAF1*	NM_002880.4	c.770C>T	p.(Ser257Leu)	Missense	LP (PS3, PM1, PM2, PP2)	rs80338796	ClinVar (VCV000013957.105): P	Exon 7	22389993, 25706034	N/A	N/A
76	*RAF1*	NM_002880.4	c.1457A>G	p.(Asp486Gly)	Missense	LP (PP3, PM2, PM5)	rs397516815	ClinVar (VCV000040618.21): P/LP	Exon 14	20683980, 17603482	+	N/A

N/A, not available; N, normal; P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance.

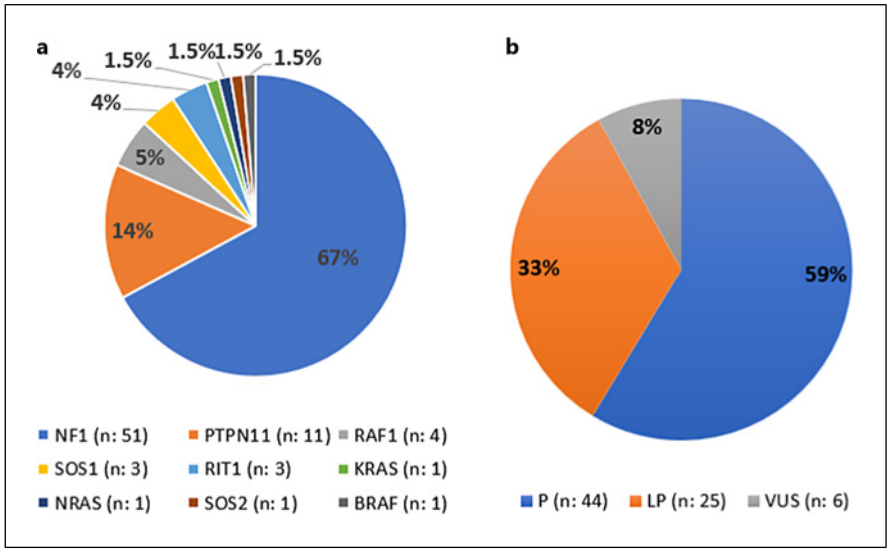

Fig. 1. a Distribution of cases based on detected gene variants. Among the index cases, 51 had *NF1* gene variants (50 different variants). There were 8 different variants in the *PTPN11* gene across 11 cases, 3 different variants in the *RAF1* gene in 4 cases, 3 variants in the *RIT1* gene, and 3 variants in the *SOS1* gene. Additionally, 1 variant each was detected in the *KRAS*, *NRAS*, *SOS2*, and *BRAF* genes. **b** Distribution of variants based on pathogenicity. When the detected variants were evaluated according to ACMG criteria, 44 were classified as pathogenic, 25 as likely pathogenic, and 6 as VUS.

Table 4. Comparison of NF1 finding frequencies with the literature

Findings	Ferner et al.[a], %	Our study, %
CALM	>99	100
Intertriginous freckling	85	86
Cutaneous/subcutaneous/plexiform neurofibroma	99/15/60	64 (all types of neurofibromas)
Lisch nodules	>95	56
Optic glioma	15	4
Sphenoid wing dysplasia	1	2
Cardiac findings	2 (12–15 in different reports)[c]	40
Scoliosis	10	41
MRI findings	68/83[b]	88
Learning disability	60	34
Seizures	6–7	5
Malignant peripheral nerve sheath tumor	2–5	2
Breast cancer	Fivefold increased risk	2

CALM, café-au-lait macule; MRI, magnetic resonance imaging. [a][15]. [b][16, 17]. [c][18, 19].

overgrowth, and premature thelarche. Additionally, her 50-year-old mother had a history of breast cancer; however, genetic testing could not be performed to evaluate the presence of the same deletion.

Although NF1 has complete penetrance, clinical variability can be observed even within the same family, and the diagnosis may be missed. For this reason, follow-up is recommended even when findings are mild, as additional symptoms may develop over time and lesions may increase even if there are no CALMs or only a few at birth. Additionally, the risk of malignancy and other conditions may increase with age.

Among the individuals diagnosed with NS, the most frequently identified variant was in the *PTPN11* gene, which is consistent with reports in the literature. Here, 770C>T (S257L) variant in the *RAF1* gene is associated

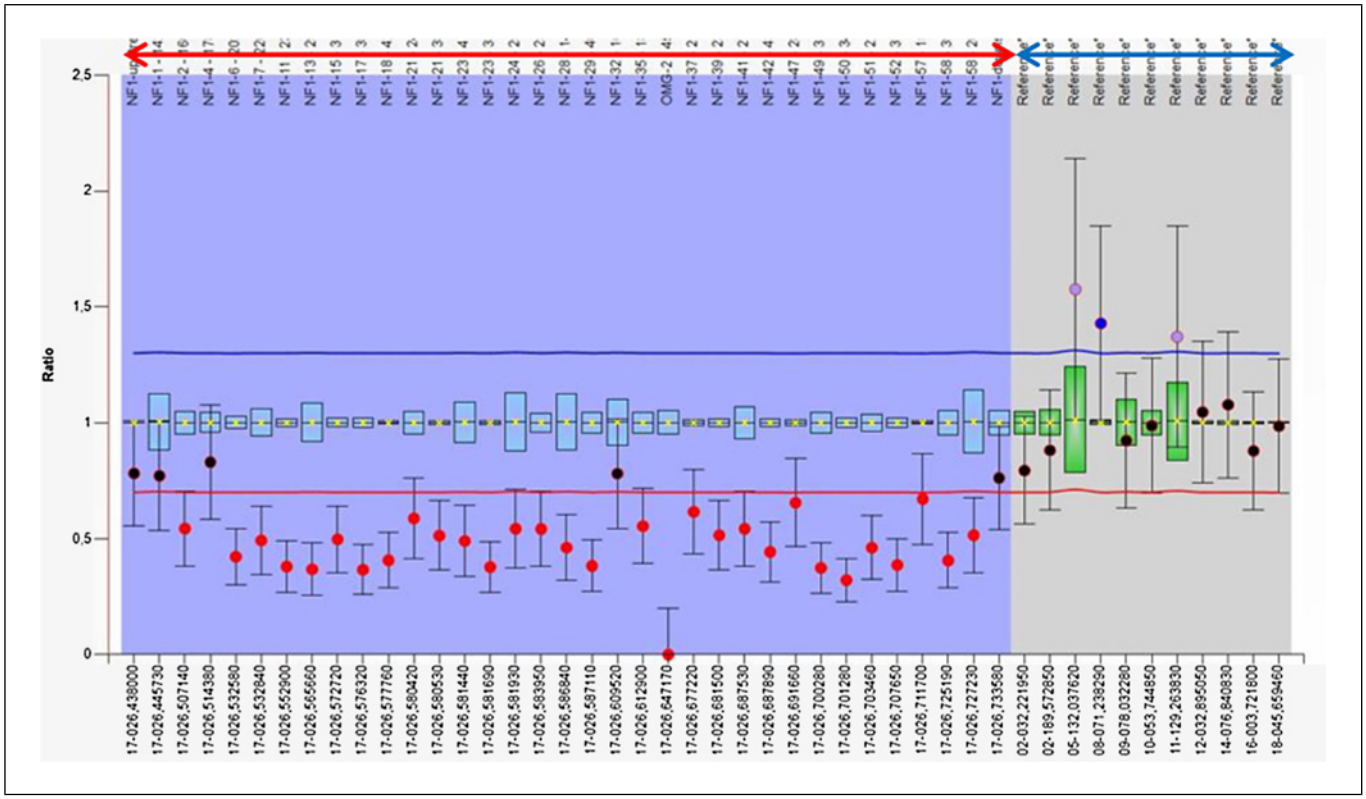

Fig. 2. MLPA analysis of the *NF1* gene for patient 43. The image demonstrates a comparison between the patient's DNA sample (indicated by the red arrow) and reference probes located on chromosomes 2, 5, 8, 9, 10, 11, 12, 14, 16, and 18 (indicated by the blue arrow). The analysis reveals a heterozygous deletion spanning exons 1–58 of the *NF1* gene when comparing the study sample to the control sample.

with both NS type 5 and NSML type 2. This variant was identified in patient 67, who was diagnosed with NSML, and in patient 75, for whom a postnatal examination was planned to confirm the diagnosis. Xu et al. [25] previously reported that this variant can lead to both NS and NSML.

Although there is no well-defined mutational hotspot within the *NF1* gene [26, 27], certain exons have been reported to be more frequently affected by pathogenic variants. In our study, variant clustering in specific exons suggests possible mutational hot spots. Most *NF1* variants (12%) were found in exon 21, a region previously associated with recurrent mutations due to its role in neurofibromin function [28]. Similarly, 36% of *PTPN11* variants were located in exon 3, aligning with reports identifying exons 3 and 13 as hotspots linked to NS via SHP2 dysfunction [29, 30]. In *RAF1*, 75% of variants clustered in exon 7 – previously associated with hypertrophic cardiomyopathy [31]. *SOS1* variants were more dispersed, reflecting its broad mutational spectrum [32]. The identification of recurrently mutated exons in our cohort highlights potential target regions for prioritized screening; however, further studies are needed to validate these findings.

In this study, 6 patients carrying VUS were included, each meeting the established clinical criteria for a RASopathy diagnosis. Although VUSs lack definitive pathogenic classification, they lie on a spectrum of evidence, and their interpretation often requires integration of phenotypic and molecular data. In most of these cases, the concordance between the clinical phenotype and known gene-disease associations, together with consistent predictions from multiple in silico tools, supported their potential diagnostic relevance. Although the variants identified in patient 66 and patient 71 were classified as VUS with relatively low pathogenicity evidence, they were included in the study as the patients fulfilled clinical RASopathy criteria and no alternative variants were detected that could explain the phenotype [33]. Nevertheless, clinical follow-up, periodic reanalysis, and extended genetic testing were planned for these cases. Recognizing the inherent

limitations of VUS classification, further assessment based on the guidelines, segregation analysis, and functional evidence would enhance the robustness of these interpretations.

Conclusion

RASopathies share several common features; yet, each syndrome within this group also exhibits unique clinical findings and distinct genetic variants that facilitate differential diagnosis. The clinical course of the disease can vary depending on the specific gene and variant identified. Therefore, both clinical correlation and molecular analysis are crucial for accurate diagnosis and management.

Given the notable familial inheritance in RASopathy cases, it is crucial to perform clinical assessments on family members and conduct segregation analysis to prevent overlooking cases with mild dysmorphic features. As new RASopathy syndromes continue to be defined, cases with suspected but undiagnosed RASopathy should undergo follow-up evaluations, comprehensive testing, and, if necessary, reanalysis to ensure accurate diagnosis.

Acknowledgments

We are grateful to the patients and their family for participating in this study.

Statement of Ethics

This study protocol was reviewed and approved by Institutional Ethics Committee, Inonu University Faculty of Medicine, dated November 16, 2021, and numbered 2021/2632. Written informed consent was obtained from the patients or legal guardians of patients for the publication of this study and any accompanying images.

Conflict of Interest Statement

The authors declare no competing interests.

Funding Sources

No financial assistance was received in support of this study.

Author Contributions

K.A. participated in the design of the study, performed physical examination of the patients and sequence analysis, and wrote the manuscript. M.O., Z.E., E.C., I.D., S.G., and B.O. performed the physical examination of the patient. M.D., A.G., H.S., B.Y., and A.F. conducted the N.G.S. analysis. I.T. contributed to the study design and analyzed the sequencing data.

Data Availability Statement

The data in this article are not publicly available because of concerns regarding patient anonymity. Requests to access the data should be directed to the corresponding author.

References

1 Riller Q, Rieux-Laucat F. RASopathies: from germline mutations to somatic and multigenic diseases. Biomed J. 2021;44(4):422–32. https://doi.org/10.1016/j.bj.2021.06.004
2 Tidyman WE, Rauen KA. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. Curr Opin Genet Dev. 2009;19(3):230–6. https://doi.org/10.1016/j.gde.2009.04.001
3 Fowlkes JL, Thrailkill KM, Bunn RC. RASopathies: the musculoskeletal consequences and their etiology and pathogenesis. Bone. 2021;152:116060. https://doi.org/10.1016/j.bone.2021.116060
4 Rauen KA. The rasopathies. Annu Rev Genomics Hum Genet. 2013;14:355–69. https://doi.org/10.1146/annurev-genom-091212-153523
5 Rodríguez F, Gaete X, Cassorla F. Etiology and treatment of growth delay in Noonan syndrome. Front Endocrinol. 2021;12:691240. https://doi.org/10.3389/fendo.2021.691240
6 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. Genet Med. 2015;17(5):405–24. https://doi.org/10.1038/gim.2015.30
7 Riggs ER, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) Elsevier; 2020.
8 Gelb BD, Cavé H, Dillon MW, Gripp KW, Lee JA, Mason-Suares H, et al. ClinGen's RASopathy Expert Panel consensus methods for variant interpretation. Genet Med. 2018;20(11):1334–45. https://doi.org/10.1038/gim.2018.3
9 Wilcox EH, Webb RF, Tshering KC, Hughes MY, Cavé H, DiStefano MT, et al. Updated ACMG/AMP specifications for variant interpretation and gene curations from the ClinGen RASopathy expert panels. Genet Med Open. 2025;3:103430. https://doi.org/10.1016/j.gimo.2025.103430
10 Walker LC, Hoya M, Wiggins GAR, Lindy A, Vincent LM, Parsons MT, et al. Using the ACMG/AMP framework to capture evidence related to predicted and observed impact on splicing: recommendations from the ClinGen SVI Splicing Subgroup. Am J Hum Genet. 2023;110(7):1046–67. https://doi.org/10.1016/j.ajhg.2023.06.002

11 Barrea C, Vaessen S, Bulk S, Harvengt J, Misson JP. Phenotype–genotype correlation in children with neurofibromatosis type 1. Neuropediatrics. 2018;49(3):180–4. https://doi.org/10.1055/s-0037-1620239
12 Rauen KA. Defining RASopathy. Dis Model Mech. 2022;15(2):dmm049344. https://doi.org/10.1242/dmm.049344
13 Allanson JE, Roberts AE Noonan syndrome Cassidy and Allanson's Management of Genetic Syndromes; 2021; p. 651–69.
14 Choi J, An S, Lim SY. Current concepts of neurofibromatosis type 1: pathophysiology and treatment. Arch Craniofac Surg. 2022; 23(1):6–16. https://doi.org/10.7181/acfs.2022.00633.
15 Stella A, Lastella P, Viggiano L, Bagnulo R, Resta N. Clinical presentation and genetic analyses of neurofibromatosis type 1 in independent patients with monoallelic double de novo closely spaced mutations in the NF1 gene. Hum Mutat. 2022;43(10):1354–60. https://doi.org/10.1002/humu.24423.
16 Jongmans M, Otten B, Noordam K, van der Burgt I. Genetics and variation in phenotype in Noonan syndrome. Horm Res. 2004; 62(Suppl 3):56–9. https://doi.org/10.1159/000080500.
17 Tartaglia M, Cordeddu V, Chang H, Shaw A, Kalidas K, Crosby A, et al. Paternal germline origin and sex-ratio distortion in transmission of PTPN11 mutations in Noonan syndrome. Am J Hum Genet. 2004;75(3):492–7. https://doi.org/10.1086/423493.
18 Adel Fahmideh M, Tettamanti G, Lavebratt C, Talbäck M, Mathiesen T, Lannering B, et al. Parental age and risk of genetic syndromes predisposing to nervous system tumors: nested case–control study. Clin Epidemiol. 2018;10:729–38. https://doi.org/10.2147/CLEP.S159183.
19 Dubov T, Toledano-Alhadef H, Bokstein F, Constantini S, Ben-Shachar S. The effect of parental age on the presence of de novo mutations–Lessons from neurofibromatosis type I. Mol Genet Genomic Med. 2016;4(4): 480–6. https://doi.org/10.1002/mgg3.222.
20 García-Díaz JD, Balsa-Vázquez J, Rodríguez-Villa A, Férriz E. Different de novo mutations in the NF1 gene in a family with neurofibromatosis type 1. Pediatr Dermatol. 2025;42(1): 136–138. https://doi.org/10.1111/pde.15734
21 Nguyen R, Mir TS, Kluwe L, Jett K, Kentsch M, Mueller G, et al. Cardiac characterization of 16 patients with large NF1 gene deletions. Clin Genet. 2013;84(4):344–9. https://doi.org/10.1111/cge.12072
22 Wang D, Wen X, Xu LL, Chen QX, Yan TX, Xiao HT, et al. Nf1 in heart development: a potential causative gene for congenital heart disease: a narrative review. Physiol Genomics. 2023;55(10):415–26. https://doi.org/10.1152/physiolgenomics.00024.2023.
23 İncecik F, Hergüner ÖM, Alınç Erdem S, Altunbaşak Ş. Neurofibromatosis type 1 and cardiac manifestations. Turk Kardiyol Dern Ars. 2015;43(8):714–6. https://doi.org/10.5543/tkda.2015.27557.
24 Kehrer-Sawatzki H, Mautner V-F, Cooper DN. Emerging genotype–phenotype relationships in patients with large NF1 deletions. Hum Genet. 2017;136(4):349–76. https://doi.org/10.1007/s00439-017-1766-y.
25 Xu S, Fan Y, Sun Y, Wang L, Gu X, Yu Y. Targeted/exome sequencing identified mutations in ten Chinese patients diagnosed with Noonan syndrome and related disorders. BMC Med Genomics. 2017;10:62–7. https://doi.org/10.1186/s12920-017-0298-6.
26 Kırat E, Albayrak HM. The spectrum of NF1 gene variations in southeastern Turkey. Spectrum. 2021;8(3):286–96.
27 Ko JM, Sohn YB, Jeong SY, Kim HJ, Messiaen LM. Mutation spectrum of NF1 and clinical characteristics in 78 Korean patients with neurofibromatosis type 1. Pediatr Neurol. 2013;48(6):447–53. https://doi.org/10.1016/j.pediatrneurol.2013.02.004
28 Upadhyaya M, Kluwe L, Spurlock G, Monem B, Majounie E, Mantripragada K, et al. Germline and somatic NF1 gene mutation spectrum in NF1-associated Malignant Peripheral Nerve Sheath Tumors (MPNSTs). Hum Mutat. 2008;29(1):74–82. https://doi.org/10.1002/humu.20601
29 Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet. 2001;29(4):465–8. https://doi.org/10.1038/ng772
30 Zenker M, Buheitel G, Rauch R, Koenig R, Bosse K, Kress W, et al. Genotype-phenotype correlations in Noonan syndrome. J Pediatr. 2004;144(3):368–74. https://doi.org/10.1016/j.jpeds.2003.11.032
31 Pandit B, Sarkozy A, Pennacchio LA, Carta C, Oishi K, Martinelli S, et al. Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. Nat Genet. 2007; 39(8):1007–12. https://doi.org/10.1038/ng2073
32 Roberts AE, Araki T, Swanson KD, Montgomery KT, Schiripo TA, Joshi VA, et al. Germline gain-of-function mutations in SOS1 cause Noonan syndrome. Nat Genet. 2007;39(1):70–4. https://doi.org/10.1038/ng1926
33 van der Burgt I. Noonan syndrome. Orphanet J Rare Dis. 2007;2:4. https://doi.org/10.1186/1750-1172-2-4