Research Article

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Title: Noonan syndrome: molecular and clinical findings in individuals with PTPN11 pathogenic variants.

Short title: Noonan syndrome with PTPN11 pathogenic variants.

Abstract

Purpose: RASopathies encompass a spectrum of disorders resulting from pathogenic variants in genes associated with the Ras/mitogen-activated protein kinase (RAS/MAPK) pathway, critical for cellular functions like proliferation, differentiation and survival. Noonan syndrome (NS), the most prevalent form of RASopathies, presents with a myriad of clinical features including characteristic facial dysmorphisms, congenital heart defects, and developmental delays. Despite its clinical recognition, molecular confirmation remains elusive in a notable percentage of cases. In this study, we aimed to investigate the clinical and molecular profiles of six patients diagnosed with NS, focusing on the role of *PTPN11* gene mutations.

Materials and methods: Molecular evaluation was performed using *PTPN11* gene sequence analysis and whole gene sequencing methods in six patients who were thought to have typical NS phenotypes based on clinical evaluations.

Results: Molecular screening in patients identified four different pathogenic variants in the *PTPN11* gene. These variants, all heterozygous, were classified as pathogenic according to established criteria.

Conclusion: Our findings contribute to understanding the genetic landscape of NS and underscore the significance of molecular analysis in confirming diagnoses.

Keywords: Noonan syndrome, *PTPN11*, pathogenic variant, SHP2.

Makale başlığı: Noonan sendromu: PTPN11 patojenik varyantları olan bireylerde moleküler ve klinik bulgular.

Öz

Amaç: RASopatiler, çoğalma, farklılaşma ve hayatta kalma gibi hücresel işlevler için kritik olan, Ras/mitojenle aktifleştirilen protein kinaz (RAS/MAPK) yolu ile ilişkili genlerdeki patojenik varyantlardan kaynaklanan, bir dizi bozukluğu kapsar. RASopatilerin en yaygın şekli olan Noonan sendromu (NS), karakteristik yüz dismorfizmleri, konjenital kalp defektleri ve gelişimsel gecikmeler dahil olmak üzere sayısız klinik özellik ile ortaya çıkar. Klinik olarak tanınmasına rağmen, vakaların kayda değer bir yüzdesinde moleküler doğrulama hala belirsizliğini korumaktadır. Bu çalışmada PTPN11 gen mutasyonu bulunan NS tanısı alan altı hastanın klinik ve moleküler profillerini araştırmayı amaçladık.

Gereç ve yöntem: Klinik değerlendirmelere göre tipik NS fenotipine sahip olduğu düşünülen altı hastada PTPN11 gen dizi analizi ve tam gen dizileme yöntemleri kullanılarak moleküler değerlendirme yapıldı.

Bulgular: Hastalarda yapılan moleküler taramada PTPN11 geninde dört farklı patojenik varyant tespit edildi. Tamamı heterozigot olan bu varyantlar, belirlenen kriterlere göre patojenik olarak sınıflandırıldı.

Sonuç: Bulgularımız NS'nin genetik yapısının anlaşılmasına katkıda bulunmakta ve tanıların doğrulanmasında moleküler analizin öneminin altını çizmektedir.

Anahtar kelimeler: Noonan sendromu, *PTPN11*, patojenik varyant, SHP2.

Introduction

RASopathies a group of diseases caused by pathogenic variants in genes encoding the Ras/mitogen-activated protein kinase (RAS/MAPK) pathway. The RAS/MAPK pathway has been associated with cell differentiation, proliferation, metabolism, cell survival and apoptosis [1]. Noonan syndrome (NS) (OMIM:163950) is the most common form (prevalence rate in livebirths of 1:1000 to 1:2500) of RASopathies, approximately 80% of its cases are associated with abnormal activation of the RAS/MAPK pathway but still 10-20% of clinical diagnoses remain unconfirmed at the molecular level [2, 3]. To date, many genes involved in the RASopathies have been identified; *PTPN11, SOS1, SOS2, KRAS, RAF1, RIT1, NRAS, BRAF, LZTR1, MAP2K1, RRAS2, RASA2, MAP2K2, HRAS, NF1, SHOC2, SPRED1, CBL, PPP1CB, MAPK1* and *MRAS* [4]. In 50% of NS cases mutation is observed in the *PTPN11* (protein tyrosine phosphatase non-receptor type 11) gene on the long arm of chromosome 12q24.1. *SOS1* (20%), *RAF1* (3-17%) and *KRAS* (<5%) mutations are less common [5, 6]. Other well-known causal genes are *RIT1*, *BRAF*, *NRAS* and *LZTR1* [7, 8, 9].

NS is a syndrome in which autosomal dominant inheritance has been reported but cases are usually sporadic [10, 11]. Mental retardation is observed in approximately 1/3 of the patients. Typical facial dysmorphism (epicanthus, ptosis, hypertelorism, downward slanting palpebral fissures, flattened nasal root, low ears, prominent upper lip, retrognathia) is the most important criterion for diagnosis. Low nape hairline and short/mane neck, raised chest, cubitus valgus syndrome are other findings that can be observed. The heart defects most commonly associated with NS are pulmonary stenosis, hypertrophic cardiomyopathy and septal defects respectively. Other clinical findings include developmental delay, chest deformities, cryptorchidism in boys, mild mental retardation, lymphatic dysplasia, bleeding diathesis and feeding difficulties in the neonatal period [12, 13].

In this study, the clinical and molecular findings of 6 patients with a clinical diagnosis of NS were evaluated and the genotype-phenotype relationship in NS was discussed.

Materials and methods

Clinical evaluation

Six cases with suspicion of Noonan Syndrome aged between 3 months and 26 years were included in this study. Criteria developed by Van der Burgt [10] were applied to all patients. Patients underwent detailed physical and clinical evaluation, including systemic examination for minor and major anomalies. Facial findings of the patients are given in Figure 1. All patients were evaluated for short stature, developmental delay, congenital heart defects, history of predisposition to bleeding, history of cryptorchidism, skeletal abnormalities, radiological test results, hearing test results, and karyotype results (Table 1).

Informed written consent was obtained from participants and their parents for photographs and genomic study. This study complies with the principles of the Declaration of Helsinki. We followed the CARE guidelines to write the case report. Written informed consent was obtained from the legal representatives of the family before blood samples were taken. All clinical tests and researches were done in Dr. Ersin Arslan Training and Research Hospital, Genetic Diseases Diagnosis Center and Pamukkale University Medical Genetics Departments.

Ethics committee approval for this study was received from Pamukkale University Non-Invasive Clinical Research Ethics Committee.

Molecular screening

DNAs were extracted from peripheral blood samples from the patients. Sequence analysis was performed using an automated capillary sequencer method in four patients. Mutations in several genes encoding components of the RAS/MAPK pathway are known to cause the N/CFC/C syndrome disease group, including 5 genes encoding components of the RAS/MAPK pathway [*PTPN11* exons 2-4, 7, 8, 11-14 (NM_002834), *KRAS* isoform B exon 2, 3, 5 (NM_004985), *RAF1* exons 7, 12, 14, 17 (NM_002880), *SHOC2* exon 2 partial (NM_007373), *SOS1* exons 3-11, 13-14, 16 (NM_005633)] exons and flanking intronic regions were amplified by PCR and analyzed by high-resolution melting on a Light Cycler (Roche, LC-480). Amplicons obtained by PCR were sequenced using an automated capillary sequencer. The sequences were compared with reference sequences in the NCBI (National Center for Biotechnology Information) database. *PTPN11* whole gene sequencing was performed on the DNA samples of the other two patients. The 16 exons of the *PTPN11* gene and their flanking intron sequences were amplified by polymerase chain reaction and sequenced with the Illumina MiSeq system. The resulting sequences were aligned to the hg19 genome using Illumina MiSeq Reporter software. Identified variants were checked against those present in 1,000 Genomes, HGMD, ClinVar and dbSNP. ACMG (American Standards and Guidelines for Medical Genetics and Genomics) criteria [14] were used for detection of variant pathogenicity.

Result

As a result of the study, 4 different pathogenic variants were detected in the *PTPN11* gene in 6 cases in which NS-related disease was considered and variant analysis was performed. All of the variants detected in the cases were heterozygous and are shown in Table 2. These variants were identified in HGMD [15] and ClinVar [16] and were interpreted as "Pathogenic" according to the ACMG criteria (PS2, PM1, PM2, PP2, PP3 criteria were applied) [14].

Discussion

Noonan syndrome is a very common disease that varies in severity and can involve more than one organ system throughout the patient's life. Approximately half of the cases are sporadic and are mostly inherited in an autosomal dominant inherited [13]. Almost 50% of Noonan patients have a heterozygous missense mutation in the *PTPN11* gene which encodes SHP2 (Src homology region 2). SHP2 is a non-receptor protein tyrosine phosphatase consisting of a catalytic PTP domain and two tandem SH2 domains (N-

terminal SH2 and C-terminal SH2) that phosphorylate tyrosine-phosphorylated signaling proteins and a C-terminal hydrophilic tail [17]. The structure and function of this protein, a member of the RAS/MAPK cascade, is evolutionarily well conserved. The majority of missense mutations in the *PTPN11* gene cause activation of the catalytic domain of the protein product that transmits excessive amounts of RAS/MAPK signals [4]. According to the literature, the exons of the *PTPN11* gene containing the most pathogenic variants are exon 3 and exon 8 constitute 62% of *PTPN11* pathogenic variants [17].

As a result of molecular genetic study, variants were detected in 6 patients. All identified variants were previously reported missense pathogenic variants and occurred at conserved positions among vertebrate *PTPN11* orthologous genes [15, 16]. Three of the detected variants are located in exon 3, one in exon 4 and two in exon 7. Exon 8 mutation (c.922A>G) which is defined as the most common mutation seen in NS, was not detected in any of our patients. All variants we found in our patients were heterozygous missense changes.

According to the literature the most frequently detected variant is the c.922A>G (p.Asn308Asp) variant [18]. In studies in our country, the exons with the highest mutation rate are exons 3 and 8 and the most frequently detected variant is c.922A>G (p.Asn308Asp) [19, 20]. On the contrary, we could not detect the c.922A>G (p.Asn308Asp) change in our patients.

To date, no significant correlation has been reported between intellectual disability and *PTPN11* pathogenic variants (the exon where the variants is located - the area in the protein it affects) in patients with NS. Only one of our patients had intellectual disability and had an exon 3 variants. Cognitive abilities and development of the other two patients with the PTPN1 gene exon 3 variant and the patients with exon 4-7 variants were found to be normal. Cryptorchidism has been reported in a range of 44-94% in male patients with Noonan syndrome [17, 18]. We detected cryptorchidism in all of our male cases.

NS is one of the syndromes without chromosomal abnormalities with Turner-like phenotypic features. Cardiovascular pathologies are reported at a rate of 40-50% [21]. The most common cardiac malformation in Noonan syndrome is valvular pulmonary stenosis due to pulmonary valve dysplasia and hypertrophic cardiomyopathy with an incidence of 37.9-39% [22]. Many chromosomal abnormalities; may manifest themselves with findings including short stature, heart defects and developmental delay. Chromosomal analysis was performed using conventional methods in all 6 patients and chromosomal abnormalities were excluded.

In conclusion, this study underscores the clinical and genetic heterogeneity of Noonan syndrome. Despite the predominance of *PTPN11* mutations, the absence of

exon 8 mutations in our cohort suggests genetic diversity. Understanding genotype-phenotype correlations aids in diagnosis and management, emphasizing the importance of comprehensive genetic screening in NS patients. Further research is warranted to elucidate the full spectrum of genetic variants contributing to NS pathogenesis.

Overall, our study contributes to advancing our understanding of NS by integrating clinical and molecular data, highlighting the complexity of this disorder and emphasizing the need for multidisciplinary approaches to diagnosis and management. Moving forward, continued research efforts aimed at unraveling the genetic and molecular basis of NS are essential for improving diagnostic accuracy, prognostication, and therapeutic interventions for affected individuals.

Conflict of interest: No conflict of interest was declared by the authors.

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Informed consent: Written informed consent was obtained from the patients.

Ethics committee approval: Permission was obtained from Pamukkale University Non-Invasive Clinical Research Ethics Committee (approval date: December 28, 2023 and approval number: E-60116787-020-468745).

Authors' contributions to the article

D.K. and K.K. constructed the main idea and hypothesis of the study. D.K. developed the theory and arranged/edited the material and method section Discussion section of the article written by D.K., T.D. and K.K. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

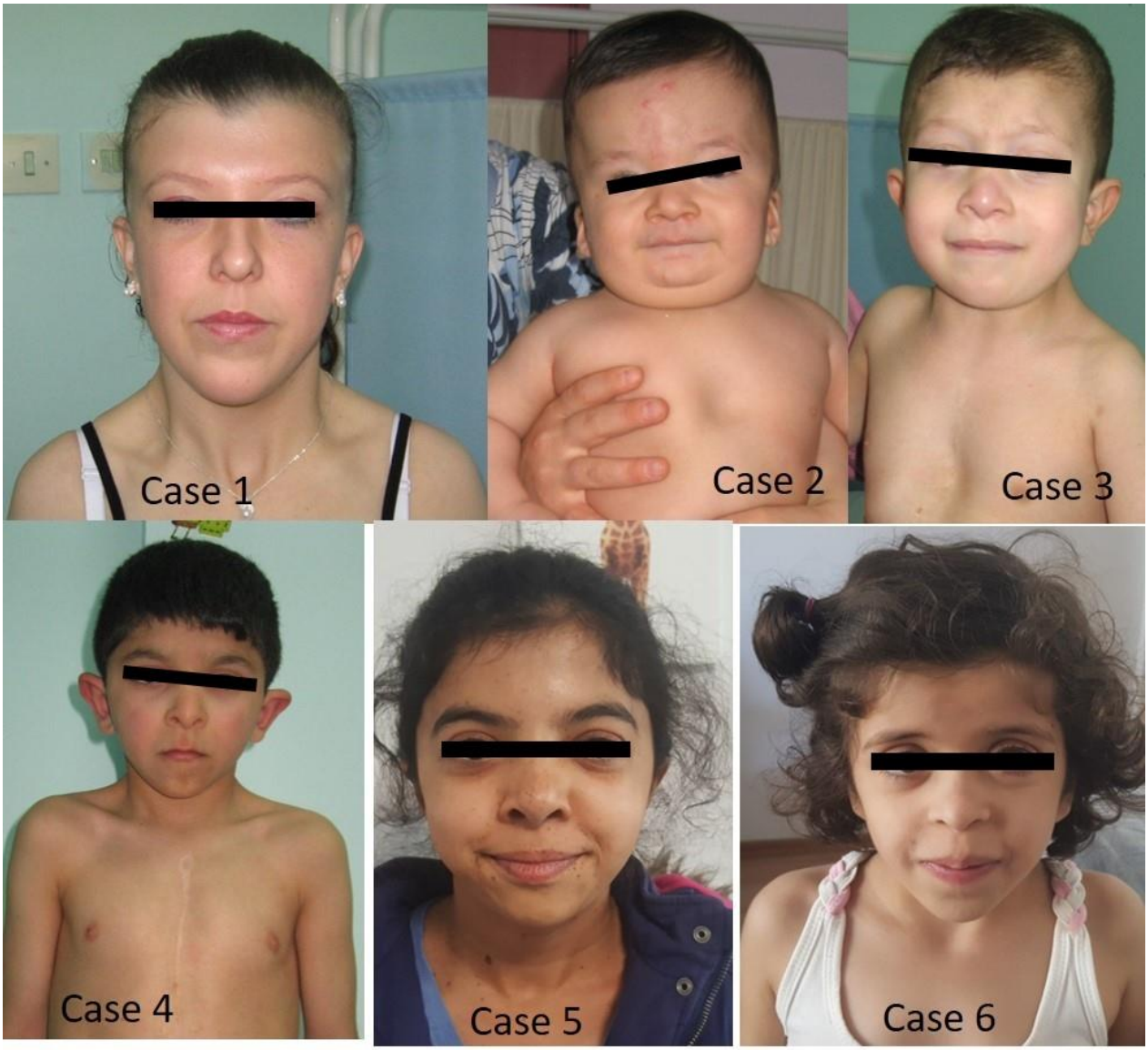

Figure 1. Facial findings of the patients

Table 1. Phenotypic and genotypic features in cases

Features	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**
Variant	*PTPN11* c.781C>T	*PTPN11* c.184T>G	*PTPN11* c.184T>G	*PTPN11* c.184T>G	*PTPN11* c.836A>G	*PTPN11* c.417G>C
Gender	F	M	M	M	F	F
Age at diagnosis	26	1	4	12	13	7
Weight (kg)	48	8,7	14	25	32	15
Height (cm)	152	68	92	124	141	112
OFD (cm)	54	45	48	50	NA	NA
Karyotype	46, XX	46, XY	46, XY	46, XY	46, XX	46, XX
Facial dysmorphism						
Ears set low and pointing backwards	+	+	+	-	-	+
Hypertelorism	-	+	+	-	+	+
Downslanting palpebral fissures	-	-	-	+	-	-
Sparse eyebrows	+	+	+	+	-	-
Prominent philtrum	+	+	+	+	+	+
Micrognathia	-	+	-	-	+	-
Triangle face	+	-	-	+	+	+
Congenital cardiac findings						
Vascular pulmonary stenosis	-	+	+	-	+	+
Ventricular septal defect (VSD)	-	-	-	-	-	-
Atrial septal defect (ASD)	+	-	+	+	-	+
Chest-neck findings						
Mane neck	+	+	+	+	-	+
Pectus deformity	+	+	+	+	+	+
Broad thorax	+	-	+	+	-	+
Bleeding disorders						
Predisposition to ecchymosis	-	-	-	-	-	-
Other findings						
Cryptorchidism	-	+	+	+	-	-
Developmental delay	-	-	+	-	-	-
Intellectual disability	-	-	+	-	-	-
Hearing loss	-	-	-	-	-	-

F: Female, M: Male

Table 2. Disease-causing variants detected in patients

Case no	Gene	Location	Functional domain of the SHP-2 protein	Nucleotid change	Amino acid change	Mutation type	Reference	Pathogenicity according to ACMG criteria
1	*PTPN11*	Exon 7	PTP	c.781C>T heterozygous	p.L261F	Missense	ClinVar, HGMD	Pathogenic
2	*PTPN11*	Exon 3	N-SH2	c.184T>G heterozygous	p.Y62D	Missense	ClinVar, HGMD	Pathogenic
3	*PTPN11*	Exon 3	N-SH2	c.184T>G heterozygous	p.Y62D	Missense	ClinVar, HGMD	Pathogenic
4	*PTPN11*	Exon 3	N-SH2	c.184T>G heterozygous	p.Y62D	Missense	ClinVar, HGMD	Pathogenic
5	*PTPN11*	Exon 7	PTP	c.836A>G heterozygous	p.Y279C	Missense	ClinVar, HGMD	Pathogenic
6	*PTPN11*	Exon 4	CSH2	c.417G>C heterozygous	p.E139D	Missense	ClinVar, HGMD	Pathogenic

HGMD: Human gene mutation database, ACMG: American College of Medical Genetics and Genomics and Association for Molecular Pathology

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