RESEARCH PAPER

Mutation and Phenotypic Spectrum of Patients With RASopathies

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Objective: To examine the common and specific clinical features, mutation spectrum and genotype-phenotype correlation in Noonan syndrome and related RASopathies. **Participants**: Records of 30 patients with clinical diagnosis of Noonan syndrome and related RASopathies presenting over a six-year period at a tertiary care medical genetics centre were reviewed. Detailed clinical phenotype evaluation and genetic testing (*PTPN11* sequencing or next generation sequencing) was done. The genetic results were used to classify the patients. **Results**: Noonan syndrome was confirmed in 22 patients, 5 had cardiofaciocutaneous syndrome and 3 had Noonan syndrome like disorder with loose anagen hair. The molecular diagnosis was confirmed in 27 patients. Mutations in *PTPN11* gene were confirmed in 57.8 % patients. Developmental delay, cardiac defects, ectodermal abnormalities and coarse face was the predominant phenotype. Noonan syndrome like disorder with loose anagen hair was clinically identifiable by the sparse, slow growing hair and caused by one recurrent SHOC2, c.4A>G mutation. **Conclusions**: Noonan syndrome and other RASopathies should be suspected in patients with short stature, cardiac defects, typical facial dysmorphism with or without ectodermal involvement.

Keywords: *Cardio-facio-cutaneous syndrome, Noonan syndrome, PTPN 11 gene, RAS/MAPK pathway.*

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RASopathies are a group of clinically defined genetic disorders with a prevalence of 1 in 1000. The patients present with a varying combination of craniofacial, cardiac, skin and skeletal phenotypes. RASopathies include neurofibromatosis type 1 (NF1), Noonan syndrome (NS), Noonan syndrome with multiple lentigines, Noonan syndrome like disorder with loose anagen hair (NSLAH), Legius syndrome, Costello syndrome (CS), cardio-facio-cutaneous syndrome (CFC) and capillary malformation arteriovenous malformation (CMAVM) [1]. All these disorders have an autosomal dominant pattern of inheritance with variable expression and penetrance.

In this study, we report on common phenotypes, diagnostic features, clinical differentiation, mutation spectrum and genotype – phenotype correlation in patients with Noonan syndrome and related RASopathies seen over a six-year period.

METHODS

In this medical record review the clinical data of patients presenting with Noonan syndrome and related disorders in our genetic clinic from 2014 through 2019 was collected on a structured defined proforma. We excluded patients with neurofibromatosis as they form a distinct, easily identifiable, group. Informed consent was taken at the time of evaluation and molecular testing from all patients/ parents included in the study. The *PTPN11* gene was sequenced or next-generation sequencing (NGS) using a panel/clinical-exome approach on Illumina HiSeq2500 was performed. All the molecular variants were classified according to the recommended method of the American College of Medical Genetics and Genomics [2]. Patients who did not undergo molecular testing were classified according to the predominant phenotype. The clinical data is represented as proportions for frequency of phenotypic features and mutations.

RESULTS

The study cohort included 30 patients, (23 males); 22 of which (16 males) were diagnosed with Noonan syndrome, five patients with CFC and three patients with NSLAH. The mean age of patients in the cohort was 7 years [range 4 months to 23 years]. Mutations were identified in 27 patients. In two patients only *PTPN11* sequencing was done, which was negative and one patient did not consent for molecular testing.

The age of diagnosis of Noonan syndrome patients ranged from 4 months to 23 years. The predominant clinical features were cardiac disease (82%), short stature (77%),

facial dysmorphism (64%), skeletal features like scoliosis, webbed neck, chest defects (pectus and wide space nipples) (45%), mild developmental delay (27%), coagulation abnormalities (23%) and cryptorchidism (14%) (**Table I**). The commonest cardiac defect was pulmonary stenosis (39%, 7/18) followed by hypertrophic cardio-myopathy (33%, 6/18). Skin features like *café au lait* macules (size varying from 5-10 mm, more than three) and hyperkeratosis were present in 27% patients. One child presented at 3 months of age with juvenile myelo-monocytic leukemia (JMML) syndrome (**Table I**). Antenatal features of cystic hygroma, bilateral choroid plexus cyst and dilated single lymphatic sac were documented in one child with Noonan syndrome related short stature.

Facial dysmorphism was present in 14 (64%) patients. (**Fig.1 a, b**). The four most characteristic features (hypertelorism, down-slanting palpebral fissures, ptosis, and low-set, posteriorly rotated ears) were present together in only four patients; 10 had atypical facies with one or two of the above dysmorphic features. Down-slanting palpebral fissures were seen in 64% and hypertelorism in 57%. Two patients had coarse facies and ectodermal features and were initially suspected as CFC syndrome but were later diagnosed as Noonan syndrome based on genetic testing (*RAF1* and *SOS2,* respectively) (**Fig. 1b**).

Table I Genotype Phenotype Correlation in 22 Patients with Noonan Syndrome

Phenotype	*N=22*	*Genes involved (genotype)*
Facial dysmorphism	14 (65%)	*RIT1* (1), *SOS2* (1) and *SOS1* with 45,
Typical gestalt, with all three cardinal features	4	X mosaic karyotype (1), *PTPN11* (1)
(hypertelorism, downslant palpebral fissure, ptosis		*PTPN11* (8)
and posteriorly rotated, low set ears)		*SOS1* (1), *RIT1* (1), *RAF* (1), *SOS1* (1),
Isolated hypertelorism	8	*PTPN11 (5)RIT1* (1),
Isolated downslant palpebral fissure	9	
Isolated ptosis	5	*SOS2* (1), *PTPN11* (3)*SOS2* (1),
Coarse facies	2	*RAF1* (1)*RAF1 (1)*
Relative macrocephaly	1	
Short stature[a]	17 (77%)	*PTPN11* (11). *SOS1* (2), *RAF1* (1),
		KRAS (1) and two mutation negative NS patients.
Cardiac defects	18 (82%)	
Pulmonary stenosis	7	*PTPN11* (3) and *SOS1 (2), RIT1* (1), *RAF1* (1)
Hypertrophic cardiomyopathy (HCM)	6	*PTPN11* (5) and *RIT1* (1)
Atrial septal defect	3	*PTPN11* (3)
Atrioventricular canal defect	1	*PTPN11 (1)*
Double outlet right ventricle with VSD	1	*SOS2 (1)*
Skeletal defects	9 (41%)	
Chest deformities (pectus, and wide space nipples)	4	*PTPN11*(3), *KRAS* (1)
Webbed neck	3	*PTPN11* (3)
Scoliosis	2	*PTPN11* (1), *SOS2* (1)
Developmental delay/intellectual disability	6 (27%)	*SOS2* (1), *RIT1* (1), *KRAS* (1),
Mild	6	*PTPN11* (3).
Cryptorchidism	3 (14%)	*PTPN11* (2), *SOS2* (1)
Renal anomalies	2 (9%)	
Vesico ureteric reflux with hydronephrosis	1	*KRAS* (1)
Renal echogenicity on fetal scan	1	*PTPN11* (1)
Coagulation abnormalities	5 (23%)	
Prolonged APTT/PT	2	*SOS1*(1), *SOS2* (1)
Factor IX deficiency	1	*PTPN11* (1)
Epistaxis	2	*PTPN11*(2)
Skin anormalites	6 (27%)	
Multiple cafe au lait	3	*PTPN11* (2), *RAF* (1)
Multiple nevi	2	*SOS1* and 45, X mosaic (1) and *PTPN11* (1)
Hyperkeratosispilaris, keloid and ulerythemaophryogenes	1	*SOS2* (1)
Juvenile myelomonocytic leukemia (JMML)	1 (5%)	*PTPN11* (1)

NS: Noonan syndrome; VSD: ventricular septal defect ; [a]Patients with SOS2 and RIT1 mutation had normal height.

Five patients of CFC syndrome were identified. All had developmental delay, coarse facies and ectodermal findings (**Web Table I**) (**Fig. 1c**). All the three patients with NSLAH had mild developmental delay, coarse facies and sparse, slow growing hair (**Fig. 1 d, e**). One patient additionally had a history of thrombotic stroke (**Web Table I**).

Of the 22 Noonan syndrome patients, mutations were present in 19 (86%) patients. These were present in *PTPN11* (11/19), *SOS1* (2/19), *SOS2* (2/19), *RIT 1* (2/19), *KRAS* (1/19) and *RAF1* (1/19) genes. The mutations and related information are listed in **Web Table II**. All the identified mutations are previously reported. The two CFC syndrome patients had the most common *BRAF* mutation, c.770A>G, p.Gln257Arg. All three NSLAH patients harbored the recurrent *SHOC2*, c.4A>G, p.Ser2Gly mutation (**Web Table I***).*

DISCUSSION

The clinical diagnosis of Noonan syndrome is traditionally on a gestalt recognition of the characteristic facial dysmorphism, cardiac malformations and short stature. Associated ectodermal features suggest CFC and NSLAH as the probable diagnosis [3,4]. In this cohort, Noonan syndrome was the commonest RASopathy (73%), followed by CFC (17%) and NSLAH (10%). The most consistent and typical facial features in the Noonan syndrome cohort were down-slanting palpebral fissures, ptosis and hypertelorism, similar to previous reports [5]. However, we also observed *PTPN11* mutation positive Noonan syndrome with atypical facies, including only hypertelorism, down-slanting palpebral fissures or ptosis. Another set of patients with mutations in uncommon Noonan syndrome genes like *RIT1, SOS1* and *SOS2* had the typical NS facial phenotype. A CFC like phenotype was seen with mutations in *RAF1* and *SOS2* associated NS suggesting a phenotypic overlap between NS and CFC. As the facial profile in NS evolves with age, it alone may be insufficient to predict the genotype, but along with other systemic features, it can aid in the clinical diagnosis [6].

The predominant cardiac lesions in NS are pulmonary stenosis (PS) and hypertrophic cardiomyopathy (HCM). Early suspicion and echocardiography is important for appropriate management as PS and HCM in *PTPN11* related NS are seldom rapidly progressive and fatal [7]. Short stature was another predominant phenotype observed in this study, which may be due to growth hormone (GH) deficiency, neurosecretory dysfunction, or GH resistance. GH therapy is approved for Noonan syndrome and should be initiated early [8].

Renal abnormalities are described in 10-11% of cases of Noonan syndrome [9]. In the present study one patient with *KRAS* associated NS (NS-3) had bilateral grade 5 vesicoureteric reflux (VUR) with hydronephrosis. VUR leading to hydronephrosis is previously unreported in Noonan syndrome. It reiterates the need for multi-organ screening in malformation syndromes for early detection and management, and prevention of related morbidity [10]. In one patient (NS-9, *SOS2* mutation) with abnormal gait and brisk deep tendon reflexes, MRI brain showed bilateral thalamic hyperintensities. MRI changes in RASopathies are previously reported, but MRI brain is recommended only if there is abnormality neurological examination [12]. Bleeding abnormalities are reported in almost 43% patients of NS while on laboratory testing abnormal coagulation profile is described in upto 90% patients [13]. One patient with NSLAH had a history of thrombotic stroke. This previously unreported association is either incidental or a disease association and needs to be addressed in additional patient cohorts. Specific *PTPN11* gene mutations predispose to an increased risk of JMML in NS patients [14], but they have a favorable prognosis and better outcomes, highlighting the importance of this

Fig. 1 Variable facial dysmorphism in Noonan syndrome: (a) Boy with Noonan Syndrome with hypertelorism, ptosis, downslant palpebral fissures, low set posteriorly rotated ears (PTPN11, exon 3, c.218C>T), (b) Boy with Noonan syndrome with cardio-facio-cutaneous syndrome like phenotype – coarse face, woolly hair, ptosis, hypertelorism, low set posteriorly rotated ears, (SOS2, Exon 6, c.800T>G), (c) Boy with cardio-facio-cutaneous syndrome - coarse face, hypertelorism, downslant eyes, low set ears, coarse hair (BRAF, exon 15, c.1802A>T) and (d), (e) Boy with Noonan syndrome like disorder with loose anagen hair - coarse face, hypertelorism, downslant eyes, relative macrocepahly and the distinct sparse slow growing hair.

WHAT THIS STUDY ADDS?

- Most Noonan syndrome patients may not have all the typical facial gestalt findings, and Hypertrophic cardiomyopathy is as prevalent as pulmonary stenosis.
- More than half of Noonan syndrome patients have mutations in exon 3, 8, 12 and 13 of *PTPN11* gene.

correlation in management protocols [15].

A previous Indian study reported exons 3 and 13 of *PTPN11* gene as the mutation hot spot in 11 Noonan syndrome patients [16]. Another study identified exons 3, 8 and 13 of *PTPN11* gene with the maximum pathogenic variants in 107 Indian patients [17]. Exons 3, 8, 12 and 13 were the hotspots exons and the commonest mutation was a previously reported, c.218C>T in exon 3 in this series. Additionally, the recurrent *SOS2*, c.800T>A mutation of NS-9 was also present in two patients [18]. We observed that most mutations in Indian patients were similar to those reported in worldwide literature.

Limitations of this study include a small number of predominantly NS patients with less representation of CFC and NSLAH. Also the absence of longitudinal follow up data limits information on management outcomes and prognosis of the patients.

Noonan syndrome should be suspected in patients with short stature (cardiac malformations, primarily pulmonary stenosis and hypertrophic cardiomyopathy), skeletal defects and facial dysmorphism (usually includes hypertelorism and down slanting palpebral fissures). *PTPN11* hot spot exon testing identifies mutations in more than half of Noonan syndrome patients.

Contributors: ML: study design, article writing, data collection; ICV: article review, critical input, study design, data collection; RDP: article critical review and writing, data collection, study design; SBM: article critical review, data collection; KM: article critical review, data collection, PTPN11 test. All authors approved the final version of manuscript.
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Web Table I Genotype Phenotype Correlation in Cardio-facio-cutaneous Syndrome (CFC) and Noonan Syndrome – Like Disorder With Loose Anagen Hair (NSLAH)

Disease	*Mutation,*	*No.*	*Age, sex*	*Face, skin and hair*	*Cardiac*	*Cognition and development*
CFC(*n*=5)	*BRAF,* exon 6, c.770A>G, commonest, CFC-1	2	1 y, F	Coarse face, Curly hair bulbous nose tip	Pulmonary stenosis	Severe developmental delay, failure to thrive, feeding difficulty developmental
			5 y, M	Coarse face, curly hair, ichthyotic skin hypopigmented facial macules	Pulmonary, stenosis Septal hypertrophy	delay, failure to thrive
	BRAF, exon 15, c.1802A>T, CFC-1	1	2 y, M	Coarse face, curly hair,		Developmental delay, failure to thrive
	MAP2K1, Exon 3 . 389A>G, Pathogenic reported CFC-3	1	6 y, M	Coarse face, curly hair , hyperkeratosis Papilloma	Atrial septal defect	Developmental delay
	MAP2K2 Exon 6, c.619G>A, likely pathogenic CFC-4	1	13 y, M	Coarse face Hyperkeratosis Curly hair Squint operated		Developmental delay, behavioral abnormalities
NSLAH (*n=3*)	*SHOC2,* c.4A>G	3	3 y, 4 y and 5 y,	Coarse facies Ptosis, sparse slow growing hair, hyper-keratosis, Café au lait spots, Thick gums, Thrombotic stroke Chin hemangioma,	Atrial septal defect (*n*=2)	Mild developmental delay

Web Table II Mutation Spectrum of Noonan Syndrome (*N*=19)

Gene	*No. of cases*	*Mutation*	*ClinVarId, Accession date – (23.5.2020)*	*OMIM*
PTPN11 (*n*=11; 56%) ENST00000351677	3	Exon 3, c.218C>T	VCV000013334.6	NS-1(163950)
	1	Exon 3, c.179G>T	VCV000055797.2	
	1	Exon 2, c.124A>G	VCV000040482.3	
	1	Exon 8, c.923A>G	VCV000013327.8	
	1	Exon 8, c.922A>G	VCV000013326.12	
	2	Exon 12, c.1403C>T	VCV000013331.11	
	1	Exon 13, c.1510A>G	VCV000040562.15	
	1	Exon 13, c.1528C>G	VCV000040566.7	
SOS1(*n*=2; 11%) ENST00000426016	2 (one Turner mosaic)	Exon 16, c.2536G>A Exon 11, c.1654A>G	VCV000040706.6 VCV000012871.7	NS-4(610733)
RIT1 (*n*=2; 11%) ENST00000368323	2	Exon 4, c.221C>G Exon 5, c.321G>A	VCV000060506.8 VCV000190305.2	NS-8 (615355)
SOS2 (*n*=2; 11%) ENST00000216373	2	Exon 6, c.800T>A Exon 6, c.800T>G	VCV000209092.3 VCV000577079.2	NS-9(616559)
KRAS (*n*=1; 6%) ENST00000311936	1	Exon 2, c.13A>G	VCV000012596.3	NS-3(609942)
RAF1 (*n*=1; 6%) ENST00000251849	1	Exon 17, c.1837C>G	VCV000013960.5	NS-5(611553)