RESEARCH BRIEF

Hotspots in *PTPN11* Gene Among Indian Children With Noonan Syndrome

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PII: S097475591600070

ABSTRACT

Objectives: To test for *PTPN11* mutations in clinically diagnosed cases of Noonan syndrome.

Methods: Seventeen individuals with clinical diagnosis of Noonan syndrome were included in the study. Sanger sequencing of all the 15 exons of *PTPN11* was done. A genotype-phenotype correlation was attempted.

Results: Mutation in *PTPN11* was detected in 11 out of 17 (64.7 %) patients with Noonan syndrome; 72% had mutation in exon 3 and 27 % had mutation in exon 13.

Conclusion: *PTPN11* mutation accounts for 64.7% of cases with clinical features of Noonan syndrome in India. Majority of the mutations are in exon 3 and exon 13 of *PTPN11*, making them the hotspots in Indian population.

Key words- Sequence analysis, mutation, protein tyrosine phosphatase non receptor type 11

Noonan syndrome (OMIM 163950) is an autosomal dominant genetic disorder with an incidence of 1 in 1,000 to 1 in 2,500 live births, [1,2] characterized by short stature, congenital heart defects, dysmorphic features and developmental delay of variable degree. *PTPN11* as the causative gene for Noonan syndrome accounts for up to 50% of cases [3,4]. *SOS1*[5], *RAF1*[6,7], *RIT1*[8], *KRAS, NRAS* ,*BRAF* and *MAP2K1* are the other genes implicated in causing the same phenotype. Mutation analysis is essential in making an accurate diagnosis and providing prenatal diagnosis. Other than some isolated case reports, mutation spectrum in Indian subjects has never been published previously. We herein present the data of 17 individuals with Noonan syndrome.

METHODS

Seventeen patients with clinical diagnosis of Noonan syndrome, based on Van der Burgt criteria [9] were included in the study. Ethical clearance was obtained from the Institute Ethics Committee. Informed consent was obtained from the patient or the parents in case of minors, for storage of blood and mutation analysis. Physical characteristics were noted and anthropometry was done in all patients. All patients underwent echocardiography for congenital heart disease. Karyotype was done in all subjects to rule out chromosomal disorders. Sanger sequencing of all the 15 exons of *PTPN11* was done. A genotype–phenotype correlation was attempted by comparing the clinical features of patients with and without mutation in *PTPN11*.

RESULTS

The age of presentation ranged from 2 months to 18 years (11 males). ***Table* I** shows the clinical features present in comparison to the mutation identified in exon 3 and exon 13 of *PTPN11*. ***Web Table* I** shows

the clinical features and the mutations identified in individual patients. The typical facial features of Noonan syndrome in subjects with a mutation identified in *PTPN11* are given in ***Web Fig.1***. Of the facial features, down slanting eyes was the consistent feature, which was present in all patients. All the patients in our cohort had short neck and short stature. The height in the study cohort ranged from -2 to -4 SD below mean.

Cardiovascular abnormalities were present in 16 (94%); the most common abnormality being pulmonary stenosis (62.5%). Echocardiography was normal in only one patient with *PTPN11* mutation.

Mild cognitive impairment was present in 6(35.2%) patients; 5 of them had a mutation in *PTPN11*.Of the 17 probands, four (25%) had other affected family members. Mutation in *PTPN11* was detected in 64.7% of patients; 8 (72%) had mutation in exon 3 and three patients had mutation in exon 13 (27%). All the variants were previously reported disease--causing variants and were reported in Human Gene Mutation Database.

DISCUSSION

Noonan syndrome is an autosomal dominant disorder with short stature, facial dysmorphism and congenital heart diseases [10]. In 20-30% of cases, disease-causing variants have not yet been identified [10].

Congenital heart disease is seen in more than 90% of Noonan syndrome, with pulmonary stenosis being the most common defect [5,10]. This finding is replicated in our study where 94% had congenital heart disease and the most common abnormality noted was pulmonary stenosis. *PTPN11* accounts for 50% of all cases of Noonan syndrome and is more prevalent in individuals with short stature and pulmonary stenosis [5]. *PTPN11* mutations have also been linked to easy bruising, pectus deformity and characteristic facial appearance [11]. In our cohort, all patients with *PTPN11* mutation had short stature, short neck and down slanting eyes. Out of these 11 patients, six (54%) had pulmonary stenosis, consistent with previous reports of pulmonary stenosis being more common in *PTPN11* mutation. As with previous studies, pulmonary stenosis remains as a marker in predicting mutation in *PTPN11* [5].

Exon 3 and exon 8 in *PTPN11* were identified as mutation hotspots in previous studies [5,12]. In our study, heterozygous variants were seen in exon 3 in 8 out of 11 individuals, comparable with previous reports. But we did not identify any variant in exon 8, but variants were found in exon 13 in two individuals. Since the sample size was limited we could not draw any definite phenotypic correlation with the exon in which mutation was identified.

We propose that exon 3 and exon 13 screening should be done in Indian subjects with short stature, downslanting eyes, short neck and pulmonary stenosis as a first step, followed by screening of other exons for variants. If no *PTPN11* mutation is identified, this should be followed by panel testing for the other genes like *SOS1, RAF1, KRAS, NRAS, SHOC2, CBL and RIT1*.Other differential diagnosis includes conditions like Costello syndrome, cardiofaciocutaneous syndrome, LEOPARD syndrome etc. Early identification and multi- disciplinary management is essential for better outcome among this group of patients. Identification of disease causing variant in a family is essential in providing prenatal diagnosis.

Contributors: DLN, HP, AM, RG, RGP, SJP: substantial contributions to the design of the work, acquisition, analysis, and interpretation of data, has drafted the manuscript, has approved version to be published and agrees to be accountable for all aspects of the work; KM, SRP: substantial contributions to the design of the work, acquisition, analysis, and interpretation of data for the work, has revised the manuscript critically and given suggestions and has approved version to be published and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding: SGPGIMS Intramural 2014-63-IMP-76

Competing interests: None stated.

WHAT THIS STUDY ADDS?

- Exon 3 and exon 13 hotspots should be checked in all Indian patients with short stature, pulmonary stenosis, down slanting eyes and short neck.

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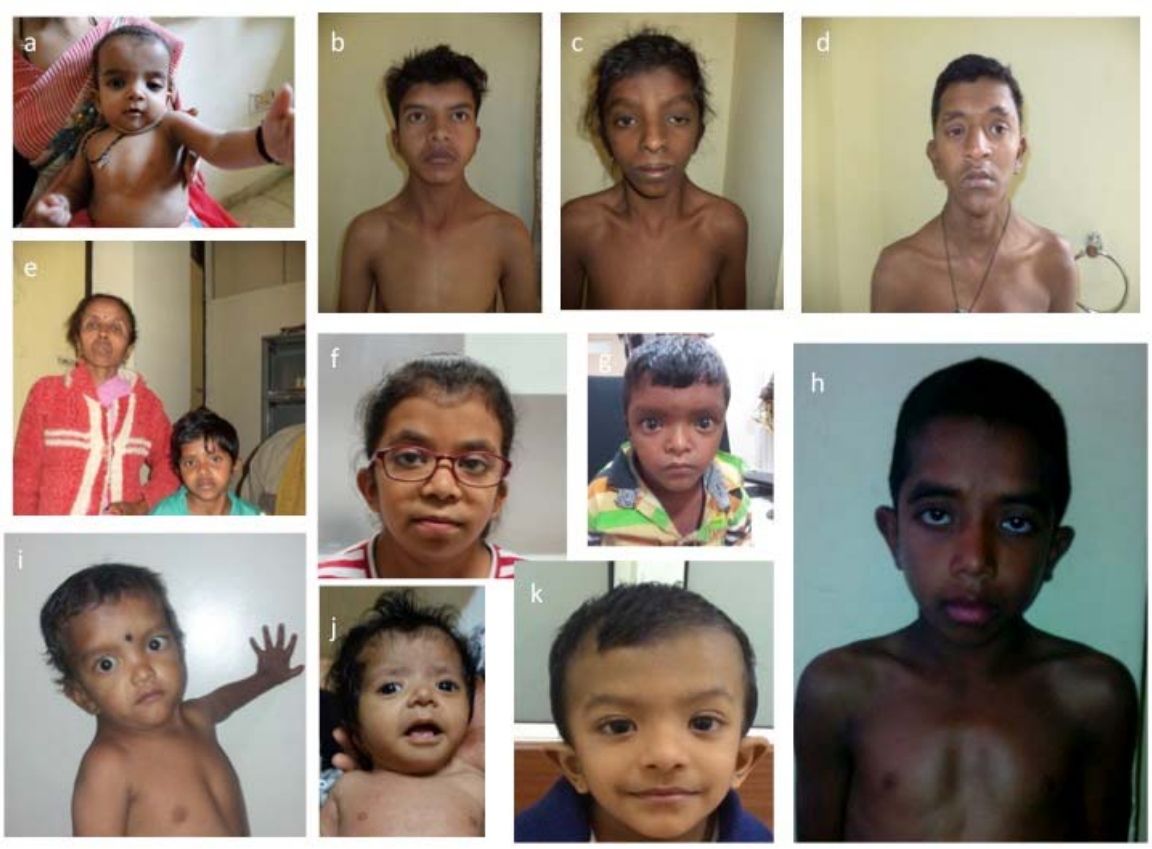
TABLE I ASSOCIATION OF CLINICAL FEATURES AND THE MUTATIONS IN *PTPN 11* IN THE STUDY CHILDREN

Clinical feature	*No.(%)*		
		PTPN11 mutation positive	
		Exon 3	*Exon 13*
Positive family history	4 (23)	2	1
Ptosis	11 (64.7)	4	2
Down slanting eyes	17 (100)	9	2
Low set ears	14 (82)	7	2
Short neck	17(100)	8	2
Pectus deformity	7(41.1)	4	1
Bleeding	1(0.05)	1	-
Congenital heart disease	16(94.1)	9	1
Short stature	17 (100)	9	2
Mild cognitive delay	6(35)	4	1

WEB TABLE I CLINICAL FEATURES AND MUTATION SPECTRUM OF PROBANDS

Label	Age	Gender	Facial Features	Congenital heart disease	Family history	Associated features	Mutation in *PTPN11*
Case 1	18 months	Female	Present	PS	No	Nil	Exon 3 c.181 G>A
Case 2	9 years	Female	Present	Severe PS	Yes	None	Exon 13, c.1510 A>G
Case 3	18 years	Male	Present	OS ASD	No	Bleeding from umbilical cord	Exon3 c.182 A>G
Case 4	13 years	Female	Present	VSD, PDA	No	None	Exon 3 c.236 A> G
Case 5	18 years	Male	Present	Normal	Yes	No	exon 13 c.1471 C>G
Case 6	6 months	Male	Present	Valvular PS	No	SNHL	exon 3 c.236 A>C
Case 7	11 years	Male	Present	Severe PS	No	None	No
Case 8	5 years	Male	Present	VSD	No	None	No
Case 9	13years	Female	Present	Mild AR,PR	No	None	Exon 13 c.1510A>C
Case 10	14 years	Male	Present	Severe Valvular PS	No	None	No
Case 11	12 years	Male	Present	Moderate PS	No	Retractile testes	exon 3, c.317 A>C
Case 12	2 months	Female	Present	OS ASD	No	None	exon 3, c.218 C>T
Case 13	2 years	Male	Present	Moderate PS, ASD	Yes	Bilateral UDT hypertelorism	exon 3, c.179 G>C
Case 14	3 years	Male	Present	Valvular PS	No	Nil	Exon 3, c.184 T>G
Case 15	30 years	Male	Present	PS	No	Scoliosis	No mutation
Case 16	8 years	Female	Present	HOCM	No	Extensive nevi	No mutation
Case 17	6 years	Male	Present	Valvular PS	Yes	Right UDT	No mutation

PS: Pulmonary stenosis, VSD: Ventricular Septal Defect, AR: Aortic regurgitation, PR: Pulmonary Regurgitation, PDA: Patent Ductus Arteriosus, OS ASD: Ostium Secundum Atrial Septal Defect, UDT: Undescended testes, HOCM: Hypertrophic Obstructive Cardiomyopathy, SNHL: Sensorineural hearing loss



***Web Fig.* 1**: Typical facial features of Noonan syndrome: (a) Down slanting eyes; (b,c,d) Short neck; (c,d) Ptosis; (k,h) low set flared ears; (i.h) Pectus excavatum.