BRIEF COMMUNICATION

Mutational Analysis of *PTPN11* Gene in Taiwanese Children with Noonan Syndrome

*Chia-Sui Hung,[1,3] Ju-Li Lin,[2,3] Yann-Jinn Lee,[4] Shuan-Pei Lin,[4] Mei-Chyn Chao,[5] Fu-Sung Lo[1,3]**

Noonan syndrome (NS) is an autosomal dominant disorder presenting with characteristic facies, short stature, skeletal anomalies, and congenital heart defects. Mutations in protein-tyrosine phosphatase, nonreceptor-type 11 (*PTPN11*), encoding SHP-2, account for 33–50% of NS. This study screened for mutations in the *PTPN11* gene in 34 Taiwanese patients with NS. Mutation analysis of the 15 coding exons and exon/intron boundaries was performed by polymerase chain reaction and direct sequencing of the *PTPN11* gene. We identified 10 different missense mutations in 13 (38%) patients, including a novel missense mutation (855T > G, F285L). These mutations were clustered in exon 3 ($n = 6$) encoding the N-SH2 domain, exon 4 ($n = 2$) encoding the C-SH2 domain, and in exons 8 ($n = 2$) and 13 ($n = 3$) encoding the PTP domain. In conclusion, this study provides further support that *PTPN11* mutations are responsible for Noonan syndrome in Taiwanese patients. [*J Formos Med Assoc* 2007;106(2):169–172]

Key Words: mutation analysis, Noonan syndrome, *PTPN11*, SHP-2

Noonan syndrome (NS) is an autosomal dominant disorder with characteristic facies, short stature, skeletal anomalies, and defects of the heart. NS was first reported by Jacqueline Noonan in 1963;[1] however, definite clinical diagnosis[2] of this condition is difficult because it is clinically heterogeneous and the phenotypic expression is highly variable and changes with age.[3] In 2001, Tartaglia et al identified the protein-tyrosine phosphatase, nonreceptor-type 11 (*PTPN11*, MIM# 176876), which encodes SHP-2, as the first NS gene.[4] Missense mutations in *PTPN11* accounted for 31–60% of cases in previous reports.[5] These mutations were demonstrated to be gain-of-function changes that resulted in excessive SHP-2 activity. In order to pinpoint the causative gene in NS in Taiwanese children, we carried out mutation screening of the *PTPN11* gene in 34 NS patients.

Methods

The study protocol was approved by the Medical Ethics and Human Clinical Trials Committee of Chang Gung Memorial Hospital. After obtaining informed parental consent, unrelated Taiwanese children with NS were enrolled. The children were screened for mutations in the *PTPN11* gene. NS was diagnosed according to major criteria (typical facial findings, cardiac defects, pterygium colli) and minor criteria (short stature, psychomotor

Divisions of [1]Pediatric Endocrinology and [2]Medical Genetics, Chang Gung Children's Hospital, [3]College of Medicine, Chang Gung University, Taoyuan, [4]Department of Pediatrics, Mackay Memorial Hospital, Taipei, and [5]Department of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

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***Correspondence to:** Dr Fu-Sung Lo, Division of Pediatric Endocrinology, Department of Pediatrics, Chang Gung Children's Hospital, 5 Fu-Shing Street, Kweishan, Taoyuan 333, Taiwan.
E-mail: lofusu@adm.cgmh.org.tw

retardation or speech delay, bleeding diathesis, family history of NS, and other additional features).[5] NS was sporadic in 30 patients and familial in four.

Genomic DNA samples were extracted from peripheral whole blood of all patients using the standard procedure. For each patient, exons 1–15 of the *PTPN11* gene were individually amplified by polymerase chain reaction (PCR) using 15 sets of primer sequences derived from published data.[6,7] The PCR products were then purified using the High Purified PCR Product Purification Kit (Roche Molecular Biochemicals, Mannheim, Germany) and directly sequenced using a cycle sequencing method.

Results

There were 34 unrelated children with NS, including 20 males and 14 females, with a mean age of 9.06 ± 5.03 years (range, 1.79–18.33 years). A total of 10 different *PTPN11* mutations, including one novel missense mutation (855T > G, F285L) (Figure), were identified in 13 (38%) patients (Table 1). All 10 mutations were heterozygous missense mutations clustered in exon 3 encoding the N-SH2 domain ($n = 6$), in exon 4 ($n = 2$) encoding the C-SH2 domain, in exon 8 ($n = 2$) or in exon 13 ($n = 3$) encoding the PTP domain. Mutations were identified in three familial cases with autosomal dominant inheritance.

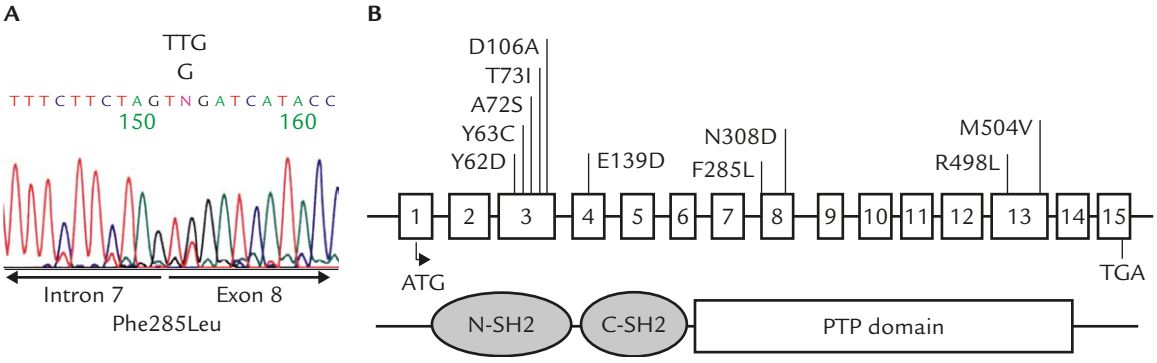

Figure. Mutation analysis of the *PTPN11* gene in Taiwanese children with Noonan syndrome. (A) Sequencing chromatogram of one novel *PTPN11* mutation (855T > G, F285L). (B) Distribution of the missense mutations identified in the *PTPN11* gene in this study.

Table 1. *PTPN11* mutations in 13 unrelated Taiwanese patients with Noonan syndrome

Case	Occurrence	Exon	Nucleotide substitution	Amino acid substitution	Functional domain	Remark
1	Sporadic	3	184A > G	Y62D	N-SH2	
2	Sporadic	3	188A > G	Y63C	N-SH2	
3	Sporadic	3	188A > G	Y63C	N-SH2	
4	Familial	3	214G > T	A72S	N-SH2	
5	Sporadic	3	218C > T	T73I	N-SH2	
6	Sporadic	3	317A > C	D106A	N-SH2	
7	Sporadic	4	417G > C	E139D	C-SH2	
8	Familial	4	417G > C	E139D	C-SH2	
9	Sporadic	8	855T > G	F285L	PTP	Novel
10	Sporadic	8	922A > G	N308D	PTP	
11	Sporadic	13	1493G > T	R498L	PTP	
12	Familial	13	1510A > G	M504V	PTP	
13	Sporadic	13	1510A > G	M504V	PTP	

This novel mutation was not found in 100 normal individuals (i.e. 200 normal chromosomes). The most frequently reported presentations of mutation-positive and -negative NS were short stature (83% *vs.* 100%), learning disabilities (82% *vs.* 71%), low hair line (77% *vs.* 71%), epicanthus (69% *vs.* 48%), webbed neck (46% *vs.* 71%), down-slanting palpebral fissures (54% *vs.* 62%), cubitus valgus (38% *vs.* 71%), thorax deformity (30% *vs.* 43%), ptosis (23% *vs.* 43%), hearing impairment (10% *vs.* 19%), cryptorchidism (17% *vs.* 47%), and pulmonary stenosis (23% *vs.* 45%). However, the prevalence of these features was not significantly different between the two groups. Atrial and/or ventricular defects were more common in the *PTPN11* mutations group than in the non-mutation group (69% *vs.* 33%, $p = 0.05$).

Discussion

This study is the first report of 10 *PTPN11* mutations in Taiwanese patients with NS. One of these was a novel mutation (855T > G, F285L), while the other nine have previously been identified in patients from other countries.[4,7] The novel mutation site was similar to that of two previously reported mutations (853T > C, F285L; 854T > C, F285S).[6,7] These findings provide further evidence that *PTPN11* mutations are responsible for the development of NS in Taiwanese.

To date, 41 different mutations have been identified in 255 individuals with NS. With the exception of two trinucleotide deletions,[8,9] the causative mutations are all missense mutations.[10] The majority of *PTPN11* mutations are recurrent and clustered in exon 3 encoding the N-SH2 domain or in exons 8 and 13 encoding the PTP domain. Shp-2, encoded by the *PTPN11* gene, has two N-terminal SH2 domains (N-SH2 and C-SH2), a classic PTP domain and a C-terminal tail. SH2 domains (particularly N-SH2) regulate PTP activity. In the basal state, Shp-2 is largely inactive because of mutual allosteric inhibition due to the "backside loop" of N-SH2 being inserted into the catalytic cleft.[11] Missense mutations in exons 3 and 8, within subregions of the N-SH2 and PTP domains, disrupt the intramolecular inhibition and lead to a conformational change resulting in a constitutively active form.

Mutations in *PTPN11* accounted for approximately 38% of NS cases in this study. This mutation detection rate is similar to findings in previous reports, with an average of 42% (255/606), ranging between 31% and 60% (Table 2).[4–7,12–15] The accumulated data suggest that *PTPN11* mutation-negative NS tend to exhibit fewer or mild clinical features of NS, even though a relatively large percentage of mutation-negative NS patients appear clinically indistinguishable from mutation-positive NS patients.[10] In this study, atrial and/or ventricular defects were more common in the mutation-positive group than in the mutation-negative group

Table 2. Summary of reported mutation rate of *PTPN11* gene in cases of Noonan syndrome to date

Authors	Year of publication	Country of publication	Frequency, *n* (%)
Targaglia et al[4]	2001	USA	11/22 (50)
Targaglia et al[7]	2002	USA	54/119 (45)
Musante et al[12]	2002	Germany	32/96 (33)
Maheshwari et al[13]	2002	USA	5/16 (31)
Kosaki et al[6]	2002	Japan	7/21 (33)
Sarkozy et al[14]	2003	Italy	23/71 (32)
Zenker et al[5]	2004	Germany	34/57 (60)
Jongmans et al[15]	2005	The Netherlands	76/170 (45)
Hung et al (this study)	2007	Taiwan	13/34 (38)
Total			255/606 (42)

(69% *vs.* 33%, $p=0.05$), a finding compatible with the results of Sarkozy et al.[14]

In conclusion, this study documented the *PTPN11* mutations and their phenotypic correlations in a series of Taiwanese patients with NS. Most patients without *PTPN11* mutation appeared clinically indistinguishable from typical *PTPN11* mutation-positive patients. Molecular analysis of the intracellular signaling mechanism of SHP-2 with clinical findings will serve to clarify the pathogenesis of NS.

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