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# A Clinical and Molecular Study of 51 Chinese Families with Noonan Syndrome

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**Abstract**

***Objective:*** To study the clinical spectrum of Chinese Noonan syndrome and perform the mutational analysis of Protein-Tyrosine Phosphatase, Non-receptor 11 (PTPN11) among them. ***Method:*** Fifty-one unrelated Noonan patients and their parents were enrolled according to a modified inclusion scoring system. In addition, Noonan-like syndromes including 2 cases of Cardio-faciocutaneous (CFC) syndrome and 1 Costello patient were tested for PTPN11 mutation. ***Results:*** Thirty-nine sporadic and 12 familial cases of Noonan syndrome were ascertained clinically. Of these familial cases, maternal versus paternal transmission occurred at a ratio of 11:1. Thirty-two index cases and 9 familial cases were found to have mutations in PTPN11 gene. Heterozygous missense mutation of PTPN11 and recurrent mutation N308D (c.922A>G) constituted 62.7% and 28.1% respectively. Five novel mutations were reported. Genotype-phenotype correlation revealed that exon 3 and 13 mutations were significantly associated with typical faces ($p<0.044$). Exon 3/c.181G>A mutation was noted to present more prominent ectodermal features like sparse hair and eyebrows. No mutation of PTPN11 gene was identified in CFC and Costello patients. ***Conclusion:*** These results demonstrate the role of PTPN11 gene in the expression of the phenotype. However, other gene(s) may also be involved in the pathogenesis of Noonan phenotype. The mutational screening of PTPN11 is recommended to ascertain those affected parents with less obvious phenotype and Noonan syndrome associated with atypical features.

**Key words** Diagnostic scoring system; Genotype-Phenotype correlation; Missense mutation; Noonan syndrome; PTPN11 gene

## Introduction

Noonan syndrome (OMIM 163950) is a well defined dysmorphic syndrome which was first reported by Kobylinski in 1883 and subsequently by Dr. Noonan JA and Ehmke DA in 1963.[1,2] It is hallmarked by a combination of distinctive faces, congenital heart defects, short stature and a wide range of developmental anomalies, namely, skeletal defects, developmental delay, cryptorchidism and bleeding diathesis. The incidence of this relatively common cardio-facial malformation is estimated to be 1 in 1000-2500 live born infants.

The cardinal facial features are easily recognised by broad and tall forehead, ptosis, hypertelorism, strabismus, downslanting palpebral fissure, low set ears, web neck and low posterior hairline. A wide range of congenital heart lesions have been reported in 90% individuals with Noonan syndrome. Stenotic dysplastic pulmonary valve and hypertrophic cardiomyopathy are the most commonly encountered cardiac lesions among those affected individuals. Though the cardio-facial anomalies defines the dysmorphic syndrome,[3] the clinicians and even dysmorphologists may be challenged by the extraordinary clinical presentations and less apparent facial manifestation

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in adult presentation. In addition, the situation is further complicated by the emergence of a variety of Noonan-like syndromes, namely, Cardio-facio-cutaneous (CFC), Costello, Multiple lentigines (ML)/Leopard, (Neurofibromatosis-Noonan) Watson, Noonan-like, short stature with sparse hair[4,5] and Noonan-like/Multiple Giant Cell Lesion syndrome.[6,7] Prior to the advances in molecular technology and the identification of PTPN11 (Protein-Tyrosine Phosphatase, Non-receptor 11) gene, they are viewed either as the variants of Noonan syndrome or some distinct clinical entities.

The Noonan syndrome is usually inherited as sporadic or autosomal dominant manner.[8] The gene was mapped to chromosome 12q and ultimately the candidate gene, PTPN11, was identified to be resided in chromosome 12q24.1.[9-12] Genetic heterogeneity was well established with the existence of autosomal recessive families and evidence of linkage exclusion to the aforementioned locus.[13]

PTPN11 gene,[14] encodes the second mammalian SRC homology 2 (SH2) domain containing intracellular PTPase. This cytoplasmic signaling enzyme is crucial to regulate the response of eukaryotic cells to extracellular signals, like growth factors, cytokines and hormones by binding the SH2 domain to specific phosphotyrosine residues within protein sequences during embryonic development. The SH2-containing tyrosine phosphatase (SHP-2) consists of a tandem SRC homology domain (N-SH2 and C-SH2), one central Protein-Tyrosine Phosphatase (PTP) domain and a carboxyl terminal tail. The N-SH2 domain functions as a conformational "switch". It switches off the activity of Phosphatase by blocking the PTP domain directly. Conversely, the binding of phosphopeptide will promote the dissociation of N-SH2 from PTP domain to generate the active conformation. On the other hand, C-SH2 does not seem to play a key role in activation. Based upon numerous observations of recurrent mutations and energetic-based structural analysis, the pathogenesis of Noonan syndrome is postulated to be the result of gain of function with disruption of the interaction between N-SH2 and PTP domains.

Missense mutations in the PTPN11 gene were identified in one-half of cases being examined. As a matter of fact, the mutation detection rate of PTPN11 gene varied quite considerably from 29% to 60% which reflected upon the stringency of the diagnostic scoring system. This gene comprises 15 exons. The mutational hotspot was exon 8 c.922A>G with other mutations clustered mainly in exons 3, 8 and 13. Genotype-phenotype correlation revealed pulmonary stenosis was more prevalent in PTPN11 mutation positive group, whereas, hypertrophic cardiomyopathy was significantly associated with PTPN11 mutation negative group.

In this study, clinical evaluation and molecular analysis were conducted for Chinese patients with Noonan syndrome using a stringent scoring system based on modification of previous published system[15-18] for evaluation. This study aimed to study the clinical spectrum and mutational spectrum of PTPN11 gene in those established Noonan cases. In addition, genotype-phenotype correlation and comparison of the clinical features of PTPN11 mutation positive and negative Noonan patients would be evaluated.

## Methods

### *Patient*

All together, 82 patients with a clinical diagnosis of Noonan syndrome between 1981-2003 were retrieved from the computer data base system of Clinical Genetic Service (CGS). The details of these patients were evaluated retrospectively from the clinical files. Fifty-one index cases were enrolled in this study according to the scoring system (Table 1). Forty-five of these cases were referred from general paediatric department for assessment of dysmorphism whereas referrals from cardiac and developmental assessment institutes constituted 4 out of 51 and 2 out of 51 cases (7.8% and 3.9%) respectively. Hence, most paediatricians would suspect Noonan syndrome based upon the facial gestalt. The exclusion and inclusion criteria of Noonan syndrome were as follows:

*Exclusion Criteria*

Known Noonan-like (e.g. Cardiofaciocutaneous, Costello, LEOPARD, WATSON) syndromes would be excluded from this clinical evaluation.

*Inclusion Criteria for Definite Noonan Syndrome*

(1) Normal karyotype and either (2) or (3)
(2) Typical faces plus 1 non-facial major criterion or 2 non-facial minor criteria
(3) Suggestive faces plus 1 non-facial major criterion and 1 non-facial minor criterion or suggestive faces plus 3 non-facial minor criteria

In this study, a scoring system (Table 1) was used to pick up the typical Noonan patients in paediatric age-group. Those affected individuals who scored at least 5 out of 6

**Table 1** Diagnostic scoring system

| |
|---|
| **Craniofacial anomalies:** |
| Broad/High forehead |
| Hypertelorism |
| Ptosis |
| Downward slanting palpebral fissure |
| Low set ears/Thickened helix |
| Web Neck/Low posterior hairline |
| **(1 point for each feature)** |
| Typical faces: >=5/6; Suggestive faces: <=4/6 |
| **Non craniofacial anomalies:** |
| ***Major criteria:*** |
| First degree relative definite Noonan syndrome |
| Typical heart lesions |
| Pulmonary stenosis or |
| Hypertrophic obstructive cardiomyopathy (HOCM) |
| Skeletal characteristics |
| Short stature* or |
| Sternal deformity |
| ***Minor criteria:*** |
| First degree relatives suggestive of Noonan syndrome |
| Atypical heart lesions |
| Developmental delay/articulation defect |
| Other characteristics |
| Widely spaced nipples or |
| Cubitus valgus or |
| Cryptorchidism or |
| Easy bruising or |
| Café au lait spot or |
| Multiple lentigenes |

*: Short stature is defined as the body height of that age which is below the 3rd centile with regard to the growth standards for Hong Kong children.[19]

craniofacial anomalies were considered to have typical faces, and those who scored 4 or less of the aforementioned were considered to have suggestive faces. Of these 51 index cases, their parents were considered to be the transmitting parents if they fulfilled either one of the following criteria:

(1) Typical or suggestive faces or
(2) 1 non-facial major criterion other than "First degree relative definite Noonan syndrome" or
(3) 2 non-facial minor criteria.

Two Noonan-like syndromes were tested for PTPN11 gene mutation including 2 Cardio-Facio-Cutaneous (CFC) patients and 1 case of Costello syndrome. The first CFC patient exhibited moderate mental retardation, Noonan-like faces plus ectodermal lesions of scanty eyebrows, sparse hair over bi-temporal regions, freckles, dark and thickening skin. No heart defect was noted. Metabolic screening, serum ACTH and synthacin tests were all normal. The second case of CFC presented with severe mental retardation and complex heart lesions of pulmonary stenosis, atrial and ventricular septal defects and hypertrophic obstructive cardiomyopathy (HOCM) as well. The Costello patient revealed Noonan-like phenotype with additional features of severe mental impairment, hyperkeratosis, acanthosis nigricans and characteristic of nasal papilloma. They both had normal karyotype and FRAXA results.

### *PTPN11 Mutational Analysis*

Genomic DNA was extracted from peripheral blood specimens using QIAamp DNA mini kit (Qiagen, Germany) according to the instructions provided by the manufacturer. The entire coding sequence of the PTPN11 gene was examined by polymerase chain reaction (PCR) and direct sequencing of the coding exon (1 to 15) and the intron-exon boundaries. Primer pairs and experimental conditions used were as described by Tartaglis M et al. (2002) with slightly modifications. The reference sequence is the human PTPN11 mRNA sequence (GeneBank Accession Number NM_002834).

PCR products were purified using the QIAquick PCR purification kit (Qiagen, Germany). Mutational analysis was performed by direct sequencing of purified PCR products using the ABI BigDye Termination Sequencing Kit and ABI 3100 Capillary Array Sequence.

### *Genotype-Phenotype Correlation*

The phenotypes of Noonan patients were matched with individual exons and major domains of the PTPN11 gene by using the standard statistical method.[20,21] This analysis was performed using 2X2 contingency-table analysis. The value was considered significant if $p<0.05$.

## Results

Fifty-one index cases of Noonan syndrome including 30 males and 21 females were enrolled into this study. All of their parents were examined. Thirty-nine sporadic cases were ascertained with no parental involvement clinically. They were all Chinese ethnics in origin. The frequencies of various clinical features of the Noonan syndrome were summarised (Table 2). In the remaining 12 cases, it was confirmed by the aforementioned clinical criteria that either mother or father was affected. Of these, maternal versus paternal transmission occurred at a ratio of 11:1. The characteristics of the affected parents were listed (Table 3). All the index cases and the affected parents were tested for PTPN11 gene.

**Table 2** Frequencies of the various clinical features among Noonan syndrome (T=51)

| Clinical features | N | Percentage (%) (N/T) |
|---|---|---|
| Craniofacial anomalies | | |
| Typical | 33 | 64.7 |
| Suggestive | 18 | 35.3 |
| Short stature | 37 | 72.5 |
| Chest deformity | 33 | 64.7 |
| Heart defects | | |
| PS | 17 | 33.3 |
| HOCM | 6 | 11.8 |
| VSD | 7 | 13.7 |
| ASD | 7 | 13.7 |
| Other valvular lesions | 7 | 13.7 |
| TOF | 1 | 2.0 |
| Arrthymias | 1 | 2.0 |
| No heart lesion | 27 | 52.9 |
| Family history | 12 | 24.0 |
| Delayed development | 20 | 39.2 |
| Café au lait spots | 3 | 5.9 |
| Cryptorchidism | 8 | 15.7 |
| Cubitus valgus | 13 | 25.5 |
| Widely spaced nipples | 15 | 29.4 |

T: Total number of cases; N: Number of cases detected; PS: Pulmonary stenosis; HOCM: Hypertrophic Obstructive Cardiomyopathy; VSD: Ventricular septal defect; ASD: Atrial septal defect; TOF: Tetralogy of Fallot

### *Mutational Spectrums of PTPN11 Gene*

Mutations were identified in 32 out of 51 index patients (Table 4). Hence the detection rate was 62.7%. Of those familial cases, 9 out of 12 cases were found to have mutations in the PTPN11 gene. All mutations were missense mutations. Eighteen different point mutations were noted all together. The most common mutation in our study was N380D (c.922A>G) accounting for 28.1%. Five novel mutations were reported, namely, c.174C>A, c.417G>C, c.794G>A, c.1471C>A and c.1472C>T. Two out of the 5 novel cases were found to be familial. All the novel mutations

**Table 3** Clinical details of transmitting parents (T=12)

| Clinical features | N | Percentage%(N/T) |
|---|---|---|
| Craniofacial anomalies | | |
| Typical | 4 | 33.3 |
| Suggestive | 6 | 50.0 |
| Normal | 2 | 16.7 |
| Short stature | 10 | 83.3 |
| Low education/ poor school performance | 7 | 58.3 |
| Known heart defects | 2 | 16.7 |

T: Total number of cases; N: Number of case detected

**Table 4** Mutational spectrum of PTPN11 gene

| E | NC | A | N | D/P/M |
|---|---|---|---|---|
| 3 | 174C>A* | Asparagine to lysine | 1 | M |
| 3 | 181G>A | Aspartic acid to asparagine | 2 | D |
| 3 | 182A>G | Aspartic acid to glycine | 2 | D |
| 3 | 184T>G | Tyrosine to aspartic acid | 1 | D |
| 3 | 214G>T | Alanine to serine | 1 | D |
| 3 | 218C>T | Threonine to isoleucine | 1 | D |
| 3 | 236A>G | Glutamine to arginine | 1 | M |
| 3 | 236A>G | Glutamine to arginine | 1 | D |
| 4 | 417G>C* | Glutamic acid to aspartic acid | 2 | D |
| 4 | 392A>G | Lysine to arginine | 1 | M |
| 7 | 794G>A* | Arginine to glutamine | 1 | D |
| 8 | 854T>C | Phenylalanine to serine | 1 | D |
| 8 | **922A>G** | Asparagine to aspartic acid | 6 | D |
| 8 | **922A>G** | Asparagine to aspartic acid | 3 | M |
| 8 | 923A>G | Asparagine to serine | 1 | D |
| 8 | 923A>G | Asparagine to serine | 1 | M |
| 12 | 1403C>T | Threonine to methionine | 1 | P |
| 13 | 1471C>A* | Proline to threonine | 1 | M |
| 13 | 1472C>T* | Proline to leucine | 1 | D |
| 13 | 1502G>A | Arginine to kysine | 1 | D |
| 13 | 1510A>G | Methionine to valine | 2 | D |

E: Exon; NC: Nucleotide change; A: Amino acid; N: Number of index case; D: Denovo; P: Paternal inherited; M: Maternal inherited

*: Novel mutations

**922A>G is equivalent to N380D mutation**

clustered in exons 3, 4 and 13. Overall 78% of identified mutations were located at critical N-SH2 and PTP domains.

Regarding the patients of CFC and Costello syndromes, no mutation was identified in the PTPN11 gene.

### *Genotype-Phenotype Correlation*

Possible association between the genotype and phenotype had been studied. We identified 2 Noonan cases (subjects 3 and 4) who carried the same exon 3/c.181G>A mutation. Both of them presented to our service at early infancy with typical characteristics of Noonan syndrome. Intriguingly, they exhibited conspicuous ectodermal problems of sparse hair and scanty eyebrows.

Major clinical features of the Noonan patients were compared with regard to mutations in individual exons and major domains. The statistical analysis revealed that only exons 3 and 13 mutations were significantly associated with typical faces ($p<0.044$) (Figure 1). There was no significant difference in comparison between other clinical manifestations and the aforementioned mutational categories.

Moreover, the clinical manifestations of Noonan syndrome were compared between the groups with and without PTPN11 gene mutation. No significant difference was observed among them (Table 5).

## Discussion

To the best of our knowledge, this is the first large scale study regarding the clinical evaluation and molecular analysis of Chinese patients with Noonan syndrome. The notable feature of this study is that we have modified a scoring system to select the Noonan patients stringently.

However, there are several inherent limitations in the scoring system. Firstly, it is not universally applicable to all age groups especially those adult Noonan patients. The transmitting parents may be missed because their characteristic facial gestalt may emerge into a less apparent form.[22-24] Only 4 out of 12 (33.3%) affected parents demonstrate typical faces in this study (Table 3). Furthermore, 2 of the affected parents with known disease-causing PTPN11 mutations have normal facial features (16.7%) on clinical assessment though they present other features of short stature and cardiac defect.

Secondly, it is noted that 2 transmitting parents have normal stature. They may achieve normal stature through catch-up growth during late adolescence as previously reported.[25,26] In addition, asymptomatic medical conditions, such as small septal defects, may resolve spontaneously during childhood and adulthood. Only 2 affected parents underwent comprehensive cardiac assessment with ascertainment of septal defect and mildly thickened mitral valve leaflets respectively. The cardiac status of the other parents remains to be clarified.

Lastly, 7 affected parents have low education level and/

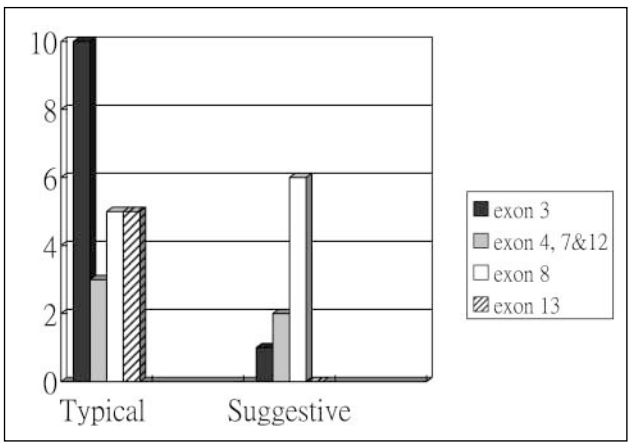


**Figure 1** Frequencies of craniofacial (Typical/Suggestive) features with different exons.

**Table 5** Clinical features of PTPN11 mutation positive and negative index patients with Noonan syndrome

| | T=32 | | t=19 | | |
|---|---|---|---|---|---|
| **Clinical features** | **N** | **%(N/T)** | **n** | **%(n/t)** | **p value** |
| Craniofacial anomalies | | | | | |
| Typical | 23 | 71.9 | 10 | 52.6 | NS |
| Suggestive | 9 | 28.1 | 9 | 47.4 | NS |
| Typical heart lesions | | | | | NS |
| PS | 13 | 40.6 | 4 | 21.1 | NS |
| HOCM | 2 | 6.3 | 4 | 21.1 | NS |
| Atypical heart lesions | 18 | 56.2 | 6 | 31.6 | NS |
| Skeletal characteristics | | | | | |
| Short stature | 25 | 78.1 | 12 | 63.2 | NS |
| Chest deformity | 23 | 71.9 | 10 | 52.6 | NS |
| Cubitus valgus | 8 | 25.0 | 5 | 26.3 | NS |
| Widely spaced nipples | 9 | 28.1 | 6 | 31.6 | NS |
| Developmental delay | 13 | 40.6 | 7 | 36.8 | NS |
| Family history | 9 | 28.1 | 3 | 15.8 | NS |
| Café au lait spot | 2 | 6.3 | 1 | 5.3 | NS |
| Cryptorchidism | 6 | 18.8 | 2 | 10.5 | NS |

T: Total number of Noonan syndrome with positive PTPN11 mutations; t : Total number of Noonan syndrome with negative PTPN11 mutations; N, n: Number of cases detected; NS: Not statistically significant; PS: Pulmonary stenosis; HOCM: Hypertrophic Obstructive Cardiomyopathy

or poor school performance. Since their phenotypic manifestations may be less obvious on clinical evaluation, the lack of proper developmental assessment may result in underestimation of their ascertainment.

The detection rate of PTPN11 gene mutation was 62.7% in this study. All of them are missense mutation. The mutational hotspot, N380D (c.922A>G), constituted 28.1% of the total PTPN11 mutation. Most (78%) mutations are located at the N-SH2 and PTP domains which reflect the importance of gain of function attributable by the disrupted interaction of these 2 key domains.

Five novel mutations are reported, namely, c.174C>A, c.392A>G, c.1471C>A, c.1472C>T and c.794G>A. Clinical details of these patients are reviewed. All of the aforementioned do not exhibit extraordinary findings.

One case of c.794G>A mutation exhibited typical Noonan phenotype, short stature, mild developmental delay, right cryptorchidism, severe myopia, failure to thrive and choledochal cyst. The presence of multiple uncommon developmental defects is postulated to be due to involvement of unraveled developmental pathways associated with PTPN11 gene. More genotype-phenotype correlation is necessary to ascertain whether choledochal cyst is associated with c.794G>A mutation.

Two cases of exon 3/c.181G>A mutation exhibit prominent ectodermal features. The underlying molecular mechanism may somehow interweave and overlap pathogenetically with the developmental pathway of SHP-1 and SHP-2. It is known that SHP-2 is widely expressed in every tissue whereas SHP-1 is limited to haematopoietic and epithelial tissues. The conspicuous ectodermal lesions in these 2 cases may be the result of exon 3/c.181G>A mutation of PTPN11 gene which specifically interact and induce the ectodermal defect in the SHP-1 pathway.

Exon 8/c.923A>G mutation was reported in some of the Noonan-like/Multiple giant-cell lesion syndrome. Cohen and Gorlin (1991) coined this as a distinctive syndrome. A familial case carrying this mutation is reported in this study. Nonetheless, they do not exhibit any features of giant-cell lesions of bone and soft tissues. Other modifying genetic factor(s) may exert concerted effort to this peculiar manifestation.

One of the Noonan patient carried exon 3/c.218C>T mutation. It has been reported that this specific mutation would predispose to the development of juvenile myelomoncytic leukaemia (JMML).[27] Indeed, specific defects in RAS, neurofibromin and PTPN11 genes will result in JMML exclusively through increased signaling of the Ras/mitogen-activated protein kinase (MAPK) cascade. The main underlying pathogenesis is thought to be the gain-of-function of these mutant alleles involving in the signal transduction.

In this study, the overall prevalence of cardiac involvement and septal defects in Noonan syndrome are 74.5% and 29.4% respectively. These findings are comparable to previous reports. However, the typical cardiac lesions of PS and HOCM are less prevalent which constituted only 33.3% and 11.8% respectively. It is speculated that other additional genetic factor(s) may play a role in the modification of cardiac presentation in the study population.

On the other hand, the PTPN11 mutation negative group constituted 19 out of 51 (37.3%) of total cases (n=51). Though previous reports have shown a higher prevalence of hypertrophic cardiomyopathy (HOCM), the findings are quite different in our study. HOCM seemed not to be a dominant heart lesion and only contributed to 21.1% (4/19). To the best of our knowledge, family history is usually negligible in this group of patients. Intriguingly, we reported 3 familial cases with all maternal transmitting parents in our study. The paramount importance of identifying these families is that genome-wide linkage analysis may aid identification of another disease locus in the future.

No PTPN11 gene mutation was identified in 2 cases of CFC syndrome and 1 Costello patient. CFC syndrome was originally described as Noonan-like facial appearance, cardiac defects, developmental delay and ectodermal anomalies.[28] Controversy exists on whether CFC and Noonan syndrome really represent 2 distinct clinical entities with overlapping features or simply a more severe clinical expression of the same allelic defects.[29,30] Lately, a report on a CFC patient with a del (12) (q21.2q22) favours the former hypothesis.[31] On the other hand, Costello syndrome has been postulated recently to be a form of metabolic storage disease.[32,33] It is postulated that the Noonan-like phenotype of Costello syndrome may result from the interaction of metabolic and PTPN11 pathways.

## Conclusion

In conclusion, the mutation detection rate of PTPN11 gene in this study was 62.7% which was compatible with other studies. For suspected Noonan syndrome in adults, apart from careful clinical assessment of the phenotype, comprehensive evaluations including proper cardiac and mental assessment, review of their childhood photographs

for evolution of phenotype and mutational screening of PTPN11 are recommended for ascertainment. It will be an economic strategy to screen N380D mutation in the first place for typical Noonan patient as it covers almost 1/3 of all the mutations. Genotype-phenotype correlation only revealed significant association between typical Noonan faces and mutations of exons 3 and 13. It is anticipated that elucidation of other Noonan syndrome gene(s) and availability of novel molecular tests will provide further insights into genotype-phenotype correlation of this syndrome.

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