ELECTRONIC LETTER

Phenotypic and genotypic characterisation of Noonan-like/multiple giant cell lesion syndrome

J S Lee, M Tartaglia, B D Gelb, K Fridrich, S Sachs, C A Stratakis, M Muenke, P G Robey, M T Collins, A Slavotinek

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Noonan-like/multiple giant cell lesion syndrome (NL/MGCLS; OMIM 163955) is a rare condition¹⁻³ with phenotypic overlap with Noonan's syndrome (OMIM 163950) and cherubism (OMIM 118400) (table 1).

Recently, missense mutations in the *PTPN11* gene on chromosome 12q24.1 have been identified as the cause of Noonan's syndrome in 45% of familial and sporadic cases,^{4,5} indicating genetic heterogeneity within the syndrome. In the study by Tartaglia *et al*,⁵ there was a family in which three members had features of Noonan's syndrome; two of these had incidental mandibular giant cell lesions.³ All three members were found to have a *PTPN11* mutation known to cosegregate with the Noonan phenotype. This mutation, an A→G transition at position 923 in exon 8, predicting an Asn308Ser substitution within the PTP domain, was identified in an unrelated kindred with classical Noonan's syndrome. No other patients with NL/MGCLS had been evaluated for the *PTPN11* mutation.

Cherubism is caused by a missense mutation in the coding region of the *SH3BP2* gene on chromosome 4p16.3.⁶ In the study by Ueki *et al*,⁶ 12 of 15 families showed point mutations in the SH3 binding protein, SH3BP2. All seven mutations identified were on exon 9 and affected three amino acids by substitution within a six amino acid sequence. A second locus or gene has not been identified.

We present the phenotype of three sporadic cases of NS/MGCLS and the results of mutation analysis of the *PTPN11* and *SH3BP2* genes.

CLINICAL REPORTS

The clinical features of the three patients are summarised in table 2.

All patients were enrolled in the National Institutes of Health IRB approved protocols and written informed consent was obtained. The three patients were diagnosed with NL/MGCLS from their clinical findings. There was no family history of cherubism, Noonan's syndrome, congenital heart disease, or consanguinity.

METHODS

G banded karyotyping was undertaken using standard techniques. The entire *PTPN11* coding region was screened, as previously reported.⁵ Briefly, unpurified polymerase chain reaction (PCR) products were analysed by denaturing high performance liquid chromatography (DHPLC), using the Wave DNA fragment analysis system (Transgenomics, Omaha, Nebraska, USA) at column temperatures recommended by the WaveMaker version 4.1.31 software (Transgenomics). Heterozygous templates with previously identified mutations were used as positive controls for all exons. Amplimers having abnormal denaturing profiles were purified (Microcon PCR, Millipore, Bedford, Massachusetts, USA) and sequenced bidirectionally using the ABI BigDye terminator sequencing kit v.3.1 (Applied Biosystems, Foster

Key points

- Noonan-like/multiple giant cell lesion syndrome (NL/MGCLS) has clinical similarities with Noonan's syndrome and cherubism. It is unclear whether it is a distinct entity or a variant of Noonan's syndrome or cherubism.
- Three unrelated patients with NL/MGCLS were characterised, two of whom were found to have mutations in the *PTPN11* gene, the mutation found in 45% of patients with Noonan's syndrome. None of the patients had a mutation of the *SH3BP2* gene known to cause cherubism.
- Giant cell lesions are likely to be a part of the spectrum of findings in Noonan's syndrome and not a distinct entity.

City, California, USA) and an ABI Prism 310 genetic analyser (Applied Biosystems). Sequencing results were analysed using the Sequencing Analysis v.3.6.1 (Applied Biosystems) and AutoAssembler v.2.1 software packages (Applied Biosystems). Mutation analysis of the *SH3BP2* gene was carried out as previously published.⁶

RESULTS

G banded karyotype analysis was normal in all three patients at a 550 band resolution. The entire coding sequences of the *PTPN11* and *SH3BP2* genes were screened by DHPLC analysis and direct sequencing. *PTPN11* mutation screening identified different heterozygous missense mutations in patients 1 and 3 (fig 3). The former was an A→C transition at position 317 in exon 3, resulting in the Asp106Ala substitution within the N-SH2/C-SH2 linker. Patient 3 showed a T→C transition at position 853 in exon 7, predicting a Phe285Leu substitution within the PTP domain. Both mutations were de novo (fig 3) and had been documented previously among individuals with Noonan's syndrome.⁵ No mutation within the *SH3BP2* gene was identified in any of the patients.

DISCUSSION

We report three unrelated patients with NL/MGCLS, two with *PTPN11* mutations and none with *SH3BP2* gene changes. The presence of these mutations supports the previous assertion that NL/MGCLS is an extreme phenotype of Noonan's syndrome. The failure to detect a *PTPN11* mutation in the third subject suggests that NL/MGCLS, like Noonan's syndrome, is genetically heterogeneous. While the promoter

Abbreviations: NL/MGCLS, Noonan-like/multiple giant cell lesion syndrome

Table 1 Comparison of the clinical characteristics of cherubism, Noonan's syndrome, and Noonan-like/multiple giant cell lesion syndrome			
System	Cherubism	Noonan's syndrome	NL/MGCLS
Inheritance	Autosomal dominant	Autosomal dominant	Autosomal dominant or sporadic
Head/neck	Full face Hypertelorism Enlarged neck lymph nodes Prognathism, malocclusion Oligodontia Giant cell lesions (maxilla, mandible, rib)	Triangular face Hypertelorism Ptosis, downward palpebral fissures Epicanthic folds Myopia Blue-green irides Low set, posterior ears; deafness Deeply grooved philtrum High arched palate Malocclusion, micrognathia Webbed neck Cystic hygroma	Giant cell lesions in bone/soft tissue Hypertelorism Prominent, posterior ears Short webbed neck Ptosis, downward palpebral fissures Epicanthic folds Full face High arched palate Malocclusion Low anterior/posterior hairlines Enlarged submandibular lymph nodes Bitemporal narrowing
Growth	–	Short stature Failure to thrive in infancy	Short stature
Cardiovascular	–	Septal defects Pulmonary stenosis Patent ductus arteriosus	Pulmonary stenosis Aortic regurgitation
Genitourinary	–	Hypogonadism Cryptorchidism	Cryptorchidism
Skeletal	–	Vertebral abnormalities Cubitus valgus Clinodactyly, brachydactyly Pectus carinatum/excavatum	Cubitus valgus Pectus carinatum/pectus excavatum Clinodactyly Generalised osteopenia
Dermatological	–	Multiple lentigines Lymphoedema Woolly hair	Multiple lentigines Café au lait spots Involut ed haemangioma
Neurological	–	Articulation difficulties Mental retardation (25%) Malignant schwannoma	Developmental delay
Haematological	–	Thrombocytopenia von Willebrand's disease Part deficiency of factor XI:C, XII:C, XIII:C	Clinically non-significant increase in PT/PTT

NL/MGCLS, Noonan-like/multiple giant cell lesion syndrome; PT, prothrombin time; PTT, partial thromboplastin time.

and enhancer regions were not examined, the nature and functional effects of the *PTPN11* lesions observed in Noonan's syndrome and NL/MGCLS make the possibility of a mutation in those non-coding regions highly unlikely.

PTPN11 encodes the non-receptor protein tyrosine phosphatase, SHP-2 (src homology region 2-domain phosphatase-2).⁷ SHP-2 is essential in multiple intracellular signal transduction pathways that affect but are not limited to mesodermal patterning and limb development,^{8,9} epidermal growth factor receptor signalling,¹⁰ and cardiac semilunar valvogenesis.¹¹ The highly conserved functional domains of the SHP-2 protein comprise two tandemly arranged SH2 domains at the N terminus (N-SH2 and C-SH2) followed by a catalytic protein tyrosine phosphatase (PTP) domain, and a carboxy-terminal tail.^{12,13} In the inactive conformation of this structure, N-SH2 and PTP interact through multiple hydrogen bonds and polar interactions blocking the PTP active site.^{14–17}

In the study by Tartaglia *et al*,⁵ most of the missense mutations affected the amino acids located in the N-SH2 and PTP functional domains, with the majority of these mutations directly involved in or located near the interacting region. This distribution suggests that the pathogenic mechanism involves an altered N-SH2/PTP interaction that destabilises the inactive conformation without altering SHP-2 catalytic capability. Molecular modelling and the first functional data support a model in which *PTPN11* mutations upregulate SHP-2 physiological activation by impairing the

switch between the active and inactive conformation, favouring a shift in the equilibrium toward the active conformation and a gain of function.^{4,18,19}

Figure 1 Multinucleated giant cells (mngc) within a fibrous stroma (f) in mandibular or maxillary bone (b). This is histologically identical to the giant cell lesions found in patients with cherubism. (H&E stain, original magnification ×20.)

Table 2 Phenotype of three patients with Noonan-like/multiple giant cell lesion syndrome

	Patient 1 (13 year old male of Italian descent)	Patient 2 (9 year old female of German descent)	Patient 3 (10 year old male of Italian descent)
Birth weight	75–90th centile	75–90th centile	75–90th centile
Birth length	>90 th centile	90th centile	>90th centile
Findings at birth	Pulmonary stenosis; bilateral cryptorchidism (underwent orchidopexy)	Pulmonary stenosis	Pulmonary stenosis, aortic regurgitation
Diagnosis (fig 1)	Age 4, noted to have facial enlargement; underwent jaw biopsy: cherubism	Age 4, noted to have facial enlargement; underwent jaw biopsy: cherubism	Age 8, routine dental x ray showed radiolucent lesion in lower jaw; underwent biopsy: cherubism
Development	Normal, except decreased height; age 11, developed diplopia/ proptosis from expanding giant cell lesions in maxilla; underwent decompression to correct vision, no sequelae; pathology report consistent with cherubism	Normal, two extremity fractures with normal healing	Mild developmental delay, requires speech therapy
Physical examination	Weight: 10–25th centile Height: 3rd centile	Weight: 25–50th centile Height: 75th centile	Weight: 10th centile Height: 25th centile
Face (fig 2)	Marked fullness; slanting palpebral fissures, hypertelorism; ICD: 37 mm (32.8 (2.8) mm); IPD >97th centile; epicanthic folds, low set ears with cupping; normal hearing; right post-auricular involution haemangioma (2×3 cm); low anterior and posterior hairline; narrow maxilla, high palatal arch, large anterior open bite, retrognathic mandible, bilateral submandibular lymphadenopathy; short neck	Marked lower facial fullness; slanting palpebral fissures, lid ptosis; low set, posteriorly angulated ears; normal hearing; 1.5×2 cm involuted haemangioma on posterior neck; low anterior hairline; large anterior open bite, prognathic mandible, high palatal arch, narrow maxilla; bilateral submandibular lymphadenopathy	Very mild lower facial fullness; slanting palpebral fissures, lid ptosis low set, posteriorly angulated ears with moderate cupping, normal hearing; low posterior and anterior hairline; high palatal arch, hypernasal speech, short neck
Chest	Prominent A-P dimension, mild inferior pectus excavatum, II/VI systolic ejection murmur at left sternal border	Moderate superior pectus carinatum, mild inferior pectus excavatum, increased inter nipple distance; II/VI systolic ejection murmur at left sternal border; multiple lentiginos on trunk, back; café au lait spot on abdomen	Mild inferior pectus excavatum, II/VI systolic ejection murmur, multiple nevi on back, café au lait spot on chest (4×6 cm)
Extremities	Bilateral cubitus valgus, fifth digit clinodactyly	Fourth/fifth digit clinodactyly, lentiginos hands/arms	Bilateral cubitus valgus, fifth digit clinodactyly
Genitals	Normal	Normal	Normal
Laboratory tests	CBC, serum chemistries (chem20), liver function tests, thyroid function tests, parathyroid hormone, urinary/serum bone markers normal in all 3 patients		
Ca, ion (1.17 to 1.31)	1.34 mmol/l	1.34 mmol/l	Normal
PTT (23.4 to 34.5 s)	38.5 s	37.2 s	45.5 s
PT (11.8 to 14.7 s)	15.7 s	14.1 s	17 s
Factor levels	Normal	Normal	Normal
Radiology	Bone age was consistent with chronological age for all 3 patients		
Skeletal survey	Generalised osteopenia	Generalised mild osteopenia	Generalised osteopenia
Face CT	Radiolucent lesions in maxilla, mandible	Radiolucent lesions in mandible	Radiolucent lesions in mandible
z Score*	–3.0 lumbar spine (DEXA scan)	–3.1 lumbar spine (DEXA scan)	–2.03 lumbar spine (qCT densitometry)
t Score†	–7.0 distal radius (DEXA scan)		
	–2.2 proximal femur (DEXA scan)		

*z Score: standard deviation of the average bone mass to age matched controls.
†t Score: standard deviation of the average peak bone mass to a young normal population, not age matched controls.
CBC, complete blood count; CT, computed tomography; DEXA, dual energy x ray absorptiometry; ICD, intercanthal distance; PT, prothrombin time; PTT, partial thromboplastin time; qCT, quantitative computed tomography.

There does not appear to be a mutation that is consistent with the presence of giant cell lesions in these patients. The identification of three *PTPN11* mutations (exon 3, Asp106Ala in patient 1; exon 7, Phe285Leu in patient 3; exon 8, Asn308Ser⁵) in cases of Noonan's syndrome with and without multiple giant cell lesions suggests that additional events may contribute to such phenotypic heterogeneity. This may include second hits in the same gene or in genes coding for signalling molecules with a role in transduction pathways in which SHP-2 is involved. Though the actual mechanism of SHP-2 in giant cell development and lesion formation is unclear, there is evidence that it is important in myeloid cell proliferation and differentiation. Gain of function somatic mutations of *PTPN11* have been identified in patients with juvenile myelomonocytic leukaemia with or without

Noonan's syndrome, myelodysplastic syndromes, and acute myeloid leukaemia, conditions in which malignant transformation has affected the myeloid precursor cells.¹⁸
All three of the patients in this study had pulmonary stenosis confirmed by echocardiography. In a review of published reports and including the three study patients, there are 24 reported cases of NL/MGCLS, of which 17 (70.8%) had pulmonary stenosis.^{1 3 20–24} In Noonan's syndrome, over 80% of the patients have a cardiovascular abnormality, pulmonary stenosis being the most common defect.^{25 26} The high prevalence of pulmonary stenosis within the NL/MGCLS population suggests that *PTPN11* will be the dominant mutated gene in this syndrome. In the study by Tartaglia *et al*,⁵ pulmonary stenosis was the most common cardiac defect and in the affected cases 70.6% had a mutation

Figure 2 Frontal views of the three patients diagnosed with Noonan-like/multiple giant cell lesion syndrome. Signed permission was obtained from the parents for the reproduction of these photographs.

in the *PTPN11* gene ($p = 0.008$). The frequent presence of cardiac defects in NL/MGCLS decreases the likelihood that it is a separate entity from Noonan's syndrome.

The three patients were all found to have low bone density compared with age matched control data. To our knowledge, low bone density has not been described previously in either NL/MGCLS, Noonan's syndrome, or cherubism. However, generalised hypomineralisation was mentioned in the case report by Cohen and Gorlin¹ of a patient with features of Noonan's syndrome and giant cell lesions. The clinical history of our three patients did not reveal an increased fracture rate. Lesions in the craniofacial region appear to be caused primarily by expansion of the cells of the bone marrow stromal compartment, and low bone mass may reflect the effects of the mutation on these cells in the appendicular bones.

In these sporadic cases of NL/MGCLS, the giant cell lesions are identical to those of cherubism by histology and clinical presentation. However, the mutations in the cherubism gene, *SH3BP2*, are absent in these patients. As mutations in the Noonan's syndrome gene are known at this time, and were found in two of the three patients, it is likely that the giant

cell lesions are a part of the spectrum of findings in Noonan's syndrome and not a distinct entity. The diagnosis of Noonan's syndrome continues to expand, and its clinical features now include giant cell lesions. However, it is unclear what additional pathogenic factors result in the formation of these giant cell lesions.

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Authors' affiliations

J S Lee, P G Robey, M T Collins, Craniofacial and Skeletal Diseases Branch, NIDCR/NIH/DHHS, Bethesda, Maryland, USA

M Tartaglia, Dipartimento di Biologia Cellulare e Neuroscienze, Istituto Superiore di Sanita, Rome, Italy

M Tartaglia, B D Gelb, Departments of Pediatrics, Mount Sinai School of Medicine, New York, USA

B D Gelb, Departments of Human Genetics, Mount Sinai School of Medicine, New York

K Fridrich, Department of Cell Biology (LHRRB), Harvard Medical School, Boston, Massachusetts, USA

S Sachs, Department of Oral and Maxillofacial Surgery, State University of New York

C A Stratakis, Developmental Endocrinology Branch, NICHD/NIH/DHHS

M Muenke, A Slavotinek*, Medical Genetics Branch, NHGRI/NIH/DHHS

*Current address: Department of Pediatrics, UCSF, San Francisco, California, USA

Conflicts of interest: none declared

Correspondence to: Dr Janice S Lee, Department of Oral and Maxillofacial Surgery, UCSF, 521 Parnassus Avenue C522, San Francisco, CA 94143-0440, USA; jslee@itsa.ucsf.edu

REFERENCES

- Cohen MM, Gorlin RJ. Noonan-like/multiple giant cell lesion syndrome. *Am J Med Genet* 1991;**40**:159–66.
- Cohen MM, Ruvalcaba RHA, Graham CB, Harrison MT, Morgan AF. A new syndrome simulating the Noonan syndrome, the Leopard syndrome, and hyperparathyroidism. *Syndrome Ident* 1974;**2**:14–17.
- Bertola DR, Kim CA, Pereira AC, Mota GF, Krieger JE, Vieira IC, Valente M, Loreto MR, Magalhaes RP, Gonzalez CH. Are Noonan syndrome and Noonan-like/multiple giant cell lesion syndrome distinct entities? *Am J Med Genet* 2001;**98**:230–4.
- Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, van der Burgt I, Crosby AH, Ion A, Jeffery S, Kalidas K, Patton MA, Kucherlapati RS, Gelb BD. Mutations in *PTPN11*, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. *Nat Genet* 2001;**29**:465–8.
- Tartaglia M, Kalidas K, Shaw A, Song X, Musat DL, van der Burgt I, Brunner HG, Bertola DR, Crosby A, Ion A, Kucherlapati RS, Jeffery S, Patton MA, Gelb BD. *PTPN11* mutations in Noonan syndrome: molecular

Figure 3 Direct sequencing and DHPLC analysis identified a heterozygous missense mutation in patient 1 and 3. Patient 1 showed an Asp106Ala substitution within the N-SH2/C-SH2 linker, while a Phe285Leu substitution within the PTP domain was seen in patient 3. Both mutations were de novo.

- spectrum, genotype-phenotype correlation, and phenotypic heterogeneity. *Am J Hum Genet* 2002;**70**:1555–63.
- 6 **Ueki Y**, Tiziani V, Santanna C, Fukai N, Maulik C, Garfinkle J, Ninomiya C, doAmaral C, Peters H, Habal M, Rhee-Morris L, Doss JB, Kreiborg S, Olsen BR, Reichenberger E. Mutations in the gene encoding c-Abl-binding protein SH3BP2 cause cherubism. *Nat Genet* 2001;**28**:125–6.
 - 7 **Dechert U**, Duncan AM, Bastien L, Duff C, Adam M, Jirik FR. Protein-tyrosine phosphatase SH-PTP2 (PTPN11) is localized to 12q24.1–24.3. *Hum Genet* 1995;**96**:609–15.
 - 8 **Saxton TM**, Henkemeyer M, Gasca S, Shen R, Rossi DJ, Shalaby F, Feng GS, Pawson T. Abnormal mesoderm patterning in mouse embryos mutant for the SH2 tyrosine phosphatase Shp-2. *Embo J* 1997;**16**:2352–64.
 - 9 **Saxton TM**, Ciruna BG, Holmyard D, Kulkarni S, Harpal K, Rossant J, Pawson T. The SH2 tyrosine phosphatase shp2 is required for mammalian limb development. *Nat Genet* 2000;**24**:420–3.
 - 10 **Qu CK**, Yu WM, Azzarelli B, Feng GS. Genetic evidence that Shp-2 tyrosine phosphatase is a signal enhancer of the epidermal growth factor receptor in mammals. *Proc Natl Acad Sci USA* 1999;**96**:8528–33.
 - 11 **Chen B**, Bronson RT, Klamon LD, Hampton TG, Wang JF, Green PJ, Magnuson T, Douglas PS, Morgan JP, Neel BG. Mice mutant for Egfr and Shp2 have defective cardiac semilunar valvulogenesis. *Nat Genet* 2000;**24**:296–9.
 - 12 **Stein-Gerlach M**, Wallasch C, Ullrich A. SHP-2, SH2-containing protein tyrosine phosphatase-2. *Int J Biochem Cell Biol* 1998;**30**:559–66.
 - 13 **Feng GS**. Shp-2 tyrosine phosphatase: signaling one cell or many. *Exp Cell Res* 1999;**253**:47–54.
 - 14 **Hof P**, Pluskey S, Dhe-Paganon S, Eck MJ, Shoelson SE. Crystal structure of the tyrosine phosphatase SHP-2. *Cell* 1998;**92**:441–50.
 - 15 **Barford D**, Neel BG. Revealing mechanisms for SH2 domain mediated regulation of the protein tyrosine phosphatase SHP-2. *Structure* 1998;**6**:249–54.
 - 16 **Eck MJ**, Pluskey S, Trub T, Harrison SC, Shoelson SE. Spatial constraints on the recognition of phosphoproteins by the tandem SH2 domains of the phosphatase SH-PTP2. *Nature* 1996;**379**:277–80.
 - 17 **Lee CH**, Kominos D, Jacques S, Margolis B, Schlessinger J, Shoelson SE, Kuriyan J. Crystal structures of peptide complexes of the amino-terminal SH2 domain of the Syp tyrosine phosphatase. *Structure* 1994;**2**:423–38.
 - 18 **Tartaglia M**, Niemeyer CM, Fragale A, Song X, Buechner J, Jung A, Hahnen K, Hasle H, Licht JD, Gelb BD. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. *Nat Genet* 2003;**34**:148–50.
 - 19 **Fragale A**, Tartaglia M, Wu J, Gelb BD. Noonan syndrome-associated SHP-2/PTPN11 mutants cause EGF-dependent prolonged GAB1 binding and sustained ERK2/MAPK1 activation. *Hum Mutat* 2004;**23**:267–77.
 - 20 **Betts NJ**, Stewart JC, Fonseca RJ, Scott RF. Multiple central giant cell lesions with a Noonan-like phenotype. *Oral Surg Oral Med Oral Pathol* 1993;**76**(5):601–7.
 - 21 **Levine B**, Skope L, Parker R. Cherubism in a patient with Noonan syndrome: report of a case. *J Oral Maxillofac Surg* 1991;**49**:1014–18.
 - 22 **Minisola G**, Porzio V, Ceralli F, Grillo LR, Porzio F. Polyarticular pigmented villonodular synovitis associated with multiple congenital anomalies. A case of Noonan-like/multiple giant cell lesion syndrome. *Clin Exp Rheumatol* 1996;**14**:207–10.
 - 23 **Ucar B**, Okten A, Mocan H, Erinc C. Noonan syndrome associated with central giant cell granuloma. *Clin Genet* 1998;**53**:411–14.
 - 24 **Addante RR**, Breen GH. Cherubism in a patient with Noonan's syndrome. *J Oral Maxillofac Surg* 1996;**54**:210–13.
 - 25 **Sharland M**, Burch M, McKenna WM, Paton MA. A clinical study of Noonan syndrome. *Arch Dis Child* 1992;**67**:178–83.
 - 26 **Marino B**, Digilio MC, Toscano A, Giannotti A, Dallapiccola B. Congenital heart diseases in children with Noonan syndrome: an expanded cardiac spectrum with high prevalence of atrioventricular canal. *J Pediatr* 1999;**135**:703–6.