

## BRIEF COMMUNICATION

# PTPN11 Mutations in LEOPARD Syndrome: Report of Four Cases in Taiwan

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**Background/Purpose:** LEOPARD syndrome (LS) is a rare, autosomal dominant disorder. The typical clinical presentation includes multiple lentigines and cardiac defects. Mutation analysis of the *PTPN11* gene is feasible. We report four cases of LS, which were confirmed by molecular genetic study.

**Methods:** The clinical features and mutations of the four patients were summarized.

**Results:** The diagnosis of all four patients was made when lentigines appeared during childhood. Three cases had hypertrophic cardiomyopathy. No electrocardiographic conduction abnormality was noted in any of the cases. Three patients had hypertelorism and three had short stature. Two patients, identical twins, presented with the atypical phenotype of tongue protrusion and hepatosplenomegaly at birth. Twin B had mild mental retardation. Case 4 had moderate hearing impairment. Point mutation of the *PTPN11* gene was found in all patients.

**Conclusion:** LS has typical skin manifestations. All patients should undergo a comprehensive examination, especially echocardiography and electrocardiography. The diagnosis can be confirmed by genetic study. [*J Formos Med Assoc* 2009;108(10):803–807]

**Key Words:** hypertrophic cardiomyopathy, LEOPARD syndrome, mutation, *PTPN11* protein

Multiple lentigines (ML)/LEOPARD syndrome (LS; MIM#151100), described for the first time by Gorlin et al in 1969,<sup>1</sup> is a rare disorder that is transmitted as an autosomal-dominant trait. The acronym LEOPARD was named to recall the syndrome: L, lentigines; E, electrocardiographic conduction abnormalities; O, ocular hypertelorism; P, pulmonary stenosis; A, abnormalities of genitalia; R, retardation of growth; and D, deafness (sensorineural). Voron et al<sup>2</sup> proposed diagnostic criteria for LS. According to these criteria, more than 100 cases have been described.<sup>2–5</sup>

Since 2002, a missense mutation in the *PTPN11* gene has been found in LS.<sup>6,7</sup> According to one

large study, about 90% of LS cases harbor *PTPN11* gene mutations.<sup>4,5</sup> Here, we report the clinical presentation and mutation analysis of four Taiwanese children with LS.

## Patients and Methods

### Patients

Four patients were diagnosed with LS at dermatology and/or genetic counseling clinics at National Cheng Kung University Hospital, Tainan, Taiwan. For diagnosis of LS, the presence of lentigines was required, in combination with at least one

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of the cardinal features outlined by Voron et al.<sup>2</sup> Clinical evaluation included family history, complete physical examination, hearing test, electrocardiography (ECG) and echocardiography. Peripheral blood samples were collected from the four patients. If their parents had the LS phenotype, samples were also collected for mutation analysis.

### Screening of *PTPN11*

Point mutations were identified by sequence analysis of amplified DNA that contained the *PTPN11* gene.

## Results

### Clinical presentation

There were one female and three male patients. All cases were identified initially by ML. Other clinical presentations are summarized in the Table. Cases 1 and 2 were premature identical twins. At the neonatal stage, the initial clinical presentation included protruding tongue, hepatosplenomegaly

and hypertrophic cardiomyopathy. In both twins, the severity of tongue protrusion subsided with time. Around 8 years of age, typical lentigines appeared and LS was diagnosed. The skin lesion appeared in case 3 at the age of 7 years (Figure) and 6 years in case 4. The mother of case 3 also had ML.

Ocular hypertelorism was noted in three cases. Hypertrophic cardiomyopathy (HCM), the



**Figure.** On examination, typical lentigines were evident over the face.

**Table.** Pooled clinical data for four cases

	Case 1 (Twin A)	Case 2 (Twin B)	Case 3	Case 4
Age at diagnosis	8 yr	8 yr	7 yr	6 yr
Sex	Male	Male	Female	Male
Lentigines	+	+	+	+
ECG conduction abnormalities	–	–	–	–
Ocular hypertelorism	+	+	+	–
Heart defect	HCM	HCM	Pulmonary stenosis status post balloon valvuloplasty	Left ventricular chamber hypertrophy
Short stature (body height < 3rd centile)	+	+	10th centile	+
Mental retardation	–	Mild	–	–
Deafness	Normal	Normal	Normal	Bilateral moderate hearing impairment
Other abnormalities	Tongue protrusion, hepatosplenomegaly	Tongue protrusion, hepatosplenomegaly		
<i>PTPN11</i> mutation				
Patient	Exon 12 (T468M), C1403T	Exon 12 (T468M), C1403T	Exon 12 (T468M), C1403T	Exon 12 (G464A), G1391C

ECG = electrocardiography; HCM = hypertrophic cardiomyopathy.

most common heart defect in LS, was noted in three cases. Pulmonary stenosis (PS) was noted in case 3. None of the patients had any conduction abnormality revealed by ECG. Development and growth were the two major issues for patients with LS. Three patients had short stature (height below the 3rd centile). Twin B had mild mental retardation. Case 4 suffered from moderate hearing impairment.

### ***Mutation analysis***

The point mutation T468M that changes amino acid 468 from threonine to methionine was identified in the twins, case 3 and her mother. In case 4, another point mutation, G464A, changed amino acid 464 from glycine to alanine.

## **Discussion**

### ***Lentiginos***

The phenotype of ML is the most distinct feature of LS. A French multicenter study pooled 30 cases, among which 28 had lentiginosis.<sup>4</sup> Sarkozy et al reported 30 cases and ML were also present in most cases (86%).<sup>5</sup> The lesions presented as flat, black–brown macules all over the body, but mostly on the face, neck and upper part of the trunk. They develop classically during childhood, increasing in number and darkening in color with age.<sup>8</sup> In the twins presented here, their skin lesion did not appear at birth. The diagnosis was not made until the age of 8 years when lentiginos appeared. In the literature review, the mean age at report was also around infancy and during childhood.<sup>4,5</sup>

### ***Electrocardiographic conduction and cardiac structural abnormalities***

Electrocardiographic conduction abnormalities and PS were the two major criteria proposed by Gorlin et al.<sup>3</sup> HCM was more common than PS. Sarkozy et al<sup>5</sup> reported 30 cases in which there were two cases with arrhythmia, three with PS, and 14 with HCM. Other cardiac abnormalities, including aortic stenosis,<sup>4</sup> atrioventricular canal

defect, mitral valve anomalies,<sup>5</sup> and cardiac myxoma<sup>9</sup> have been reported.

Cardiac structural and conduction abnormalities are the major determinants of mortality and morbidity in LS. Sudden cardiac death has previously been reported in patients with LS.<sup>10,11</sup> The two previously reported patients both had HCM and conduction abnormalities.

Three of our four cases had HCM. Case 3 had severe valvular PS. Conduction abnormalities in LS have been reported to occur gradually and progressively.<sup>9</sup> Fortunately, no conduction abnormality was noted during the regular follow-up of our cases.

### ***Ocular hypertelorism***

Reviewing the literature, the clinical presentation of hypertelorism is quite common, and this sign was also noted in three of our patients.

### ***Genital abnormalities***

Genital abnormalities include hypospadias and cryptorchidism.<sup>12</sup> In 1984, Colomb and Morel<sup>13</sup> reviewed 38 cases and found that 29% had abnormal genitalia (males). No genital abnormality was noted in our cases.

### ***Deafness (sensorineural)***

Sensorineural hearing loss has been documented in about 25% of patients with LS.<sup>2,12</sup> Hearing impairment can be detected early in the neonatal stage by auditory brainstem response audiometry (ABR). Our twins were diagnosed with hearing impairment at birth by ABR. During follow-up, both of their hearing was normal, as confirmed by pure-tone testing. Case 4 had bilateral moderate hearing impairment (right ear: 102 db; left ear: 87 db), and is wearing a hearing aid at present.

### ***Growth and mental retardation***

Colomb and Morel reported that 42% of LS had short stature.<sup>12,13</sup> In our patients, three had short stature (height below the 3rd centile). The height of case 4 was around the 10th centile.

Colomb and Morel reported mental retardation in 35% of cases.<sup>12,13</sup> Sarkozy et al reviewed

30 cases and eight had mental retardation.<sup>5</sup> In our cases, according to the Wechsler Primary and Preschool Scale of Intelligence-Revised (WPPSI-R), only twin B had mental retardation during follow-up.

#### *Other clinical manifestations*

The presence of tongue protrusion and hepatosplenomegaly in our twins has not been mentioned previously. The etiology of hepatosplenomegaly was investigated. No evidence of congenital infection was found. Liver biopsy showed no evidence of glycogen storage or other hepatic disease. The tongue protrusion subsided gradually with age. However, the hepatosplenomegaly persisted during follow-up.

#### *Mutations in PTPN11 gene*

The *PTPN11* gene encodes the protein tyrosine phosphatase SHP-2, which contains two Src homology 2 (SH2) domains. SHP-2 is a critical component of signal transduction for several growth factor-, hormone-, and cytokine-signaling pathways that control developmental processes and hematopoiesis, as well as energy balance and metabolism.<sup>14</sup> With the crucial role of SHP-2 in development, germline mutations in *PTPN11* have been identified in cases of LS, Noonan syndrome and hematologic malignancies, including juvenile myelomonocytic leukemia, myelodysplastic syndromes, acute myeloid leukemia,<sup>15</sup> and acute lymphoblastic leukemia.<sup>14</sup>

In 2002, two teams first reported that patients with LS also had mutations in *PTPN11*.<sup>6,7</sup> The mutations account for up to 90% of cases.<sup>4,14</sup> Among all mutations, T468M and Y279C account for the majority of cases. No specific genotype-phenotype correlation has been found. Further study is needed in Asian patients to identify if there are racial differences in the point mutations.

The point mutations T468M<sup>4,6,14</sup> and G464A<sup>5,14</sup> that were found in our cases have been reported in the literature. The mother of case 3 also had the same mutation as her daughter. In the other three cases, according to family history, we believe that the mutations occurred *de novo*.

## Conclusion

As a rare disease, LS can be identified by the typical feature of ML. For the wide spectrum of the syndrome, all patients should receive comprehensive examination, including echocardiography and ECG by cardiologists. A hearing test is also warranted. For the high prevalence of short stature and mental retardation, all patients need close follow-up by pediatricians and might require early intervention. At present, molecular analysis enables us to confirm the diagnosis and make genetic counseling and prenatal diagnosis more accurate.

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