Medical genetics

Familial cases of atypical clinical features genetically diagnosed as LEOPARD syndrome (multiple lentigines syndrome)

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Summary

Five familial cases exhibited ephelides-like multiple lentigines, and we examined three of them, a mother and two sons. All three patients presented with small dark-brown maculae on the face and neck and electrocardiographic abnormalities. These findings sufficed to fulfill the criteria for LEOPARD syndrome (multiple lentigines syndrome), although they lacked five of seven major clinical features. However, the family members presented with a webbed neck and pectus excavatum, which are more frequently seen in Turner or Noonan syndrome. Histological examination of the lentigines revealed slightly elongated rete ridges, a hyperpigmented basal layer, and melanophages in the papillary dermis. Direct sequencing of the patients' genomic DNA revealed that all three had a consistent missense mutation [c.1403C > T (p.T468M)] in the *PTPN11* gene, confirming LEOPARD syndrome with an atypical phenotype. It was suggested that LEOPARD syndrome shows a diverse phenotype but its diagnosis can be verified by mutation analysis.

Introduction

In 1936, Zeisler and Becker[1] reported on a 24-year-old female with multiple lentigines scattered on her body, pectus carinatum, ocular hypertelorism, and mandibular prognathism, which was later named LEOPARD syndrome (LS) by Gorlin *et al.*[2] LEOPARD is an acronym for the major features that characterize the syndrome: multiple *L*entigines, *E*lectrocardiographic conduction defects, *O*cular hypertelorism, *P*ulmonary stenosis, genital *A*bnormality, *R*etardation of growth, and sensorineural *D*eafness. LS is an autosomal dominant disorder that has been presented not only by dermatologists, but also by other specialists,[3–8] and is also called multiple lentigines syndrome.[2,9] The life-threatening problems in LS patients are hypertrophic cardiomyopathy and malignant tumors.[10,11]

Missense mutations in exons 7, 12, and 13 of the protein-tyrosine phosphatase, nonreceptor type 11 (PTPN11) gene, which is located on chromosome 12q24.1 and encodes the protein tyrosine phosphatase SHP2, have been found in LS;[10,12,13] all the mutations are located at the catalytic cleft of the *PTPN11* protein.[14] The SHP2 protein plays an important role in several signal transduction pathways involving several cytokines and hormones, with a particular role in the RAS-mitogen activated protein kinase pathway.[15–17] Thus, although genetic testing is not commonly performed, it is helpful for confirming a diagnosis and differentiating LS from similar diseases, such as Peutz-Jeghers syndrome, Carney syndrome, Noonan syndrome, and Turner syndrome.

We describe a family with members exhibiting multiple lentigines with less-frequent symptoms, such as a webbed neck (pterygium colli) and pectus excavatum (trichterbrust), who were genetically diagnosed as having LS.

Case report

The family consisted of three generations (Fig. 1). In the 1st generation, there were two sisters. The elder sister (70-year-old) had multiple dark-brown lentigines, mainly on the face (similar appearance to ephelides), a webbed

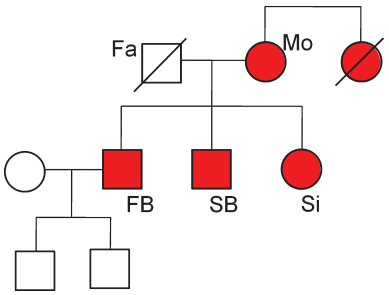

Figure 1 Family pedigree. Two family members in the 1st generation (the mother and mother's younger sister) and all three members in the 2nd generation (two sons and one daughter) presented with multiple lentigines (red). Multiple lentigines were not noted in the father and first brother's sons. Fa, father; Mo, mother; FB, first brother; SB, second brother; Si, sister

neck, and pectus excavatum without a short stature (Fig. 2). She had two sons and one daughter (the 2nd generation) and did not marry consanguineously. Her husband already died of lung cancer at the age of 64. The younger sister (65-year-old) had multiple lentigines and no children before she died.

The second brother of the 2nd generation (41-year-old) presented with small, dark brown, irregularly pigmented maculae 1 to 4 mm in size on the face and neck, including the vermillion, but not involving the oral and orbital mucosa (Fig. 2). The maculae had been present since birth, and new lesions gradually developed until his 20s and darkened with age. He also presented with other features, such as a webbed neck with a lower hairline and pectus excavatum. Electrocardiography indicated arterial fibrillation, ventricular extrasystole, tachycardia, and left anterior hemiblock. Echocardiography showed mild mitral valve regurgitation, tricuspid valve regurgitation, aorta dilation, and left ventricular dilation. Pulmonary stenosis was not found. Gastrointestinal and colon fibroscopy did not detect polyposis or any other abnormalities. Levels of thyroid stimulating hormone, free thyroxine, and free triiodothyronine were normal. Chromosome analysis showed a normal 46, XY karyotype in all the 50 peripheral lymphocytes examined. The first brother (44-year-old) (Fig. 2) and a sister (39-year-old) of the 2nd generation showed almost the same physical findings. Only the second brother had nevus spilus-like maculae on the back and left arm, but neurofibroma did not present in any of the family members. Bilateral blepharoptosis was noted also only by the second brother, although there was no accompanying exophthalmus or ocular hyperterolism.

The first brother of the 2nd generation has two sons (3rd generation), aged 6 and 5 years, with no symptoms suggesting LS, although multiple lentigines may appear in

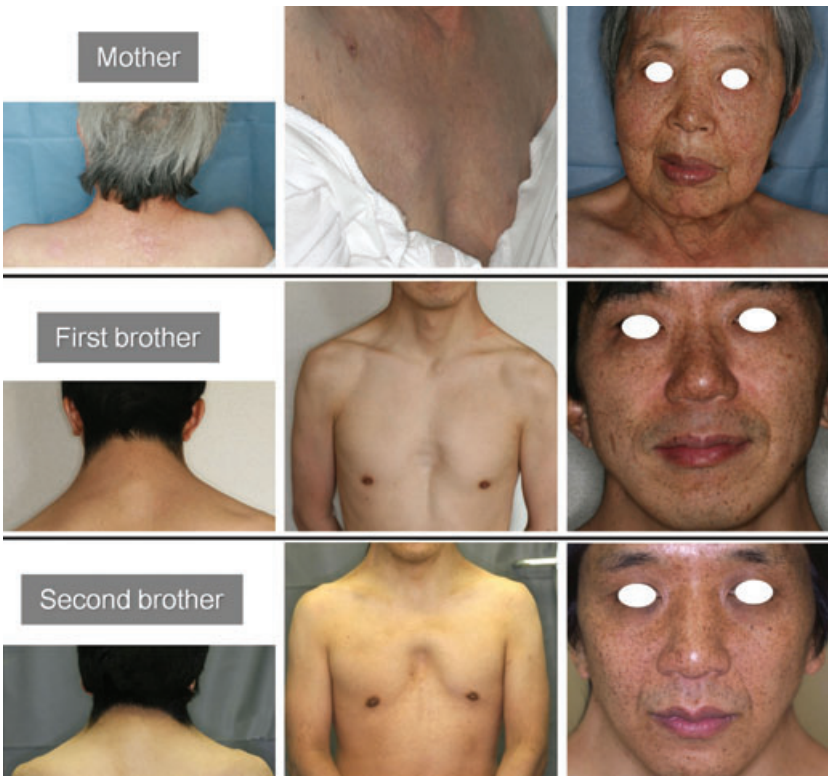

Figure 2 Photographs of three family members. All three members (the mother, the first brother and the second brother) presented with multiple small brown maculae on the face and neck, a webbed neck, and pectus excavatum

Table 1 Summarized clinical manifestations of five family members

Manifestations			Fa	Mo	FB	SB	Si
Genome	Missense mutation in the PTPN11 gene		N/A	+	+	+	N/A
L	Multiple *L*entigines		−	+	+	+	+
E	*E*CG abnormalities		N/A	+	+	+	+
O	*O*cular hypertelorism		−	−	−	−	−
P	*P*ulmonary stenosis		N/A	N/A	N/A	−	N/A
A	*A*bnormal genetalia	Cryptorchidism	−	−	−	−	−
R	*R*etardation of growth		−	−	−	−	−
D	Sensorineural *D*eafness		−	−	−	−	−
Skin	Cafè-au-lait spots		−	+	+	+	N/A
	Neurofibromatosis		−	−	−	−	−
	Curly, coarse hair		−	−	−	−	−
Ear	Low-set ear		+	+	+	+	N/A
Eye (Eyelids)	Light-colored irises		−	−	−	−	N/A
	Blepharoptosis		+	+	+	+	+
	Epicanthal folds		−	−	+	+	N/A
Cardiovascular	Congenital heart defects		N/A	N/A	N/A	+	N/A
	Hypertrophic cardiomyopathy		N/A	N/A	N/A	−	N/A
Skeletal	Short stature		−	−	−	−	−
	Pectus excavatum and/or carinatum		−	+	+	+	+
	Vertebral anomalies	Scoliosis	−	−	−	−	−
	Cubitus valgus		−	−	−	−	−
Hematological	Bleeding diathesis (von Willebrand disease, factors XI and XII deficiency)		−	−	−	−	−
	Thrombocytopenia		−	−	−	−	−
	Leukemia		−	−	−	−	−
Others	Webbed neck with low posterior hairline		−	+	+	+	+
	Malocclusion		−	+	+	+	N/A
	Lymphatic disorder	Lymphedema	−	−	−	−	−
	Triangular facies		−	−	−	−	N/A
	Feeding difficulties		−	−	−	−	−
	Cryptorchidism		−	−	−	−	−
	Mental retardation		−	−	−	−	−
	Sexual infantilism		−	−	−	−	−

ECG, electrocardiogram; Fa, father; Mo, mother; FB, first brother; SB, second brother; Si, sister.

the future. The second brother and a sister do not have any children.

There was no abnormality of the external genitalia or urinary organs in any family members. Intelligence, mental development, and hearing were also normal. The clinical data are summarized in Tables 1 and 2.

Human tissue analyses were performed in compliance with the Declaration of Helsinki Principles. Peripheral blood samples were taken from the mother (1st generation) and both brothers (2nd generation) using an ethics committee-approved protocol for genomic DNA analyses after each patient provided informed consent. Photo release consent was also obtained from each patient. Leukocyte genomic DNA was amplified by PCR for the 15 exons and flanking introns of *PTPN11* and was subjected to direct sequencing from both directions using a CEQ 8000 autosequencer (Beckman Coulter, Fullerton, CA, USA). The primer sequences and PCR conditions were

Table 2 Characteristic manifestations of LEOPARD and Noonan syndrome

Manifestations	Fa	Mo	FB	SB	Si
LEOPARD Multiple Lentigines	−	+	+	+	+
Sensorineural Deafness	−	−	−	−	−
ECG abnormalities Noonan	N/A	+	+	+	+
Facial dysmorphism (e.g. Ocular hypertelorism)	N/A	N/A	N/A	−	N/A
Cardiovascular defects (e.g Pulmonary stenosis)	−	−	−	−	−
Abnormal genetalia (e.g Cryptochidism)	−	−	−	−	−
Retardation of growth (e.g. Short stature)	−	−	−	−	−
Mental retardation	−	−	−	−	−
Webbed neck	−	+	+	+	+
Pectus excavatum	−	+	+	+	+
Hematologic abnormalities (e.g. Leukemia)	−	−	−	−	−

Fa, father; Mo, mother; FB, first brother; SB, second brother; Si, sister.

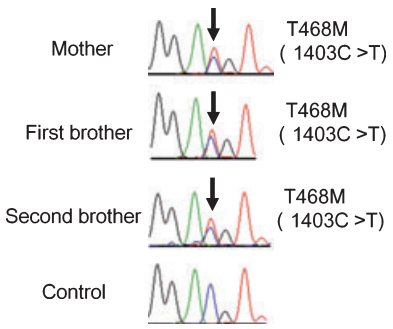

Figure 3 Electrochromatograms for the three family members. The PTPN11 mutation (Thr468Pro, 1403AfiC) was detected in genomic DNA from the leukocytes of the three patients

described previously.[18] To confirm any mutations, three independent PCR products were examined. Mutation analysis indicated a heterozygous C > T substitution at position c.1403 in *PTNP11* exon 12 in all the three subjects, resulting in the missense mutation Thr468Met (Fig. 3), which is one of the known mutations for LS. This mutation is located at the catalytic cleft of the PTP domain and impairs phosphatase activity of SHP2.[19]

A skin biopsy of a pigmented facial lesion was taken from the second brother (2nd generation). The biopsied sample was processed for HE staining and Fontana-Masson ammoniac silver staining. Histological examination of the lentigine specimen (Fig. 4) revealed that epidermal rete ridges were slightly elongated and basal layer of the epidermis were hyperpigmented with increased numbers of melanocytes. No nevus cells were observed. Deposition of melanophages was slightly detected in the top region of the dermal papillae, and we observed moderate infiltration of lymphocytes into the epidermis and hair follicle epithelium.

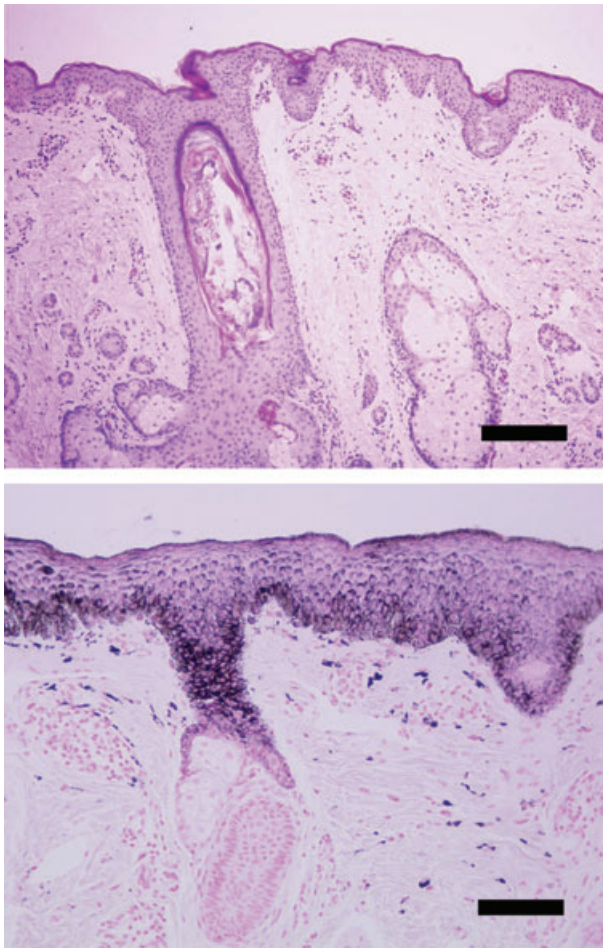

Figure 4 Histological examination of the biopsy specimen from the face of the second brother. Top: Histological examination of a pigmented macule demonstrated slightly elongated rete ridges and epidermal hypermelanosis using (Hematoxylin-Eosin staining. ×100; scale bar = 200 μm). Bottom: Higher magnification of the section revealed a hyperpigmented basal layer, incresed numbers of melanocytes without nest formation, and melanophages in the papillary dermis. (Masson-fontana ammoniac silver staining ×200; scale bar = 100 μm.)

Discussion

There are many reports in the literature of multiple lentigines associated with other symptoms, including Neurofibromatosis–Noonan syndrome,[20] Watson syndrome,[21] centrofacial lentiginosis,[22] inherited patterned lentiginosis,[23] Carney complex,[24] Peutz–Jeghers syndrome,[25] Laugier–Hunziker–Baran syndrome, and Cronkhite–Canada syndrome. In our cases, ephelides-like lentigines were spread predominantly on the face and neck without eruptions on the oral mucosa, and neither neurofibroma nor schwannoma were seen. Intestinal polyposis, myxoma, or endocrine dysfunction was not noted. However, our cases also lacked many major manifestations associated with LS; none of the patients exhibited ocular hypertelorism, pulmonary stenosis, abnormal genitalia, growth retardation, or sensorineural deafness. On the other hand, a webbed neck and pectus excavatum, which are less frequent in LS[9,26] and frequently seen in Noonan syndrome and Turner syndrome,[27] were noted.

LEOPARD syndrome has been reported to present with extremely variable phenotypes. Voron *et al.*[9] grouped the LS features into the following nine categories: cutaneous abnormalities, cardiac abnormalities, genitourinary abnormalities, endocrine findings, neurogenic defects, cephalofacial dysmorphism, short stature, skeletal anomalies, and familial history consistent with an autosomal dominant

mode of inheritance. Voron also proposed minimal diagnostic criteria for LS: at least two other features must be present in cases with multiple lentigines, whereas a diagnosis of LS may be made in cases with family history and three other major features despite an absence of multiple lentigines.[9] In our cases, three other features (cardiac and skeletal abnormalities and family history) were present in addition to multiple lentigines, but only two (multiple lentigines and ECG abnormality) of the seven major clinical manifestations advocated by Gorlin *et al.*[2] were noted. Therefore, careful differentiation from Noonan syndrome is needed because most of the clinical features of LS, such as heart defects, growth retardation, and facial dysmorphism, overlap with those of Noonan syndrome. Noonan syndrome presents as a Turner-like phenotype, such as short stature, cephalofacial dysmorphism, webbed neck, skeletal anomalies, and genitourinary and cardiac abnormalities, particularly pulmonary valve stenosis, although Noonan syndrome has a normal karyotype.[28]

Both LS and Noonan syndrome are known to be caused by heterozygous germline missense mutations in the PTPN11 gene. Approximately 85% of the patients with a definite diagnosis of LS have a missense mutation in the PTPN11 gene,[10] and mutations in the PTPN11 gene are also seen in roughly 50% of Noonan syndrome cases.[27,29] However, it was recently established by analyzing accumulated genetic data of LS and Noonan syndrome that the mutations in LS and Noonan syndrome are almost mutually exclusive.[14,30,31] In Noonan syndrome, PTPN mutations are detected at 33–60%,[27,30] and are recurrent and clustered mostly in exons 3, 7, 8, and 13.[12,27] Noonan syndrome mutations are recognized as gain-of-function mutations, while LS mutations were identified as having dominant negative, not activating, effects.[32] The most frequently (approximately 90%) reported PTPN11 mutations in LS are located in exons 7 (Tyr279Cys) and 12 (Thr468Met),[30] the latter of which was detected in all three family members examined here. In addition, to our knowledge, Thr468Met has never been detected in NS syndrome.[27,33] Taken together with the clinical finding that the three familial patients sufficed Voron's minimal diagnostic criteria for LS, we diagnosed them as LS.

It has been reported that there are typically two histological types of lentigines seen in LS patients:[9,26] melanocytic nevi and lentigo simplex. The biopsy specimen from our case exhibited histological features compatible with the latter, a lack of nevus cells and the presence of epidermal hypermelanosis.

In conclusion, three familial cases presented with ECG abnormalities and multiple lentigines on the face and neck, lacked most of other major features of LS, and exhibited a webbed neck and pectus excavatum. Genetic testing revealed that all of the patients carry a consistent germline missense mutation (Thr468Met, 1403C → T) in the exon 12 of PTPN11 gene, which suggested the diagnoses of LS.

Acknowledgment

The authors acknowledge Ms. Ayako Kurata for her technical assistance for the histological assessment. There is no funding to declare in this study.

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