Phenotypical diversity in three family members with LEOPARD syndrome caused by Tyr279Cys mutation in PTPN11 gene

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ABSTRACT

LEOPARD syndrome (LS) is an inherited autosomal dominant disorder caused by mutations in the PTPN11, RAF1 and BRAF genes. Its major characteristics include: multiple Lentigines, Electrocardiographic conduction abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retardation of growth and sensorineural Deafness. In this paper, we report the cases of three family members from two generations presenting with multiple lentigines and café-au-lait spots. Direct sequencing of the patients' genomic DNA revealed that all three had a recurrent mutation in the PTPN11 gene (c.836A>G / p.Tyr279Cys), confirming LEOPARD syndrome. Our paper shows the variable expressivity of the syndrome despite point mutation identified in our patients.

Key words : Multiple lentigines, Café-au-lait spots, LEOPARD syndrome, PTPN11 gene mutation

RESUME

Le syndrome de LEOPARD est une affection héréditaire rare qui se transmet selon le mode autosomique dominant. Elle est due à des mutations des gènes PTPN11, RAF1 and BRAF . Les principales anomalies sont résumées dans l'acronyme LEOPARD: Lentigines multiples, anomalies de conduction à l'ECG, troubles Oculaires, sténose Pulmonaire, Anomalie des organes génitaux, Retard de croissance et surdité neurosensorielle. Dans cet article, nous rapportons les cas de trois membres d'une famille sur deux générations présentant des lentigines multiples et des taches café-au-lait. Le séquençage direct de l'ADN génomique des patients a révélé une mutation récurrente du gène PTPN11 (c.836A>G/ p.Tyr279Cys) pour les 3 cas, confirmant le syndrome de LEOPARD.

Mots clés : Multiples lentigines, Tâches café-au-lait, Syndrome de LEOPARD, Mutation du gène PTPN11

INTRODUCTION

LEOPARD syndrome is an heterogeneous disorder affiliated with the family of neuro-cardiofacio-cutaneous syndromes, inherited as an autosomal dominant trait with full penetrance and variable expressivity [1]. It is the phenotypic expression of missense mutations in the protein tyrosine phosphatase nonreceptor-type 11 (PTPN11) gene, encoding protein SHP2 [2]. This protein plays the main role in several signal transduction pathways, mainly the RAS-mitogen activated protein kinase (MAKP), which are important for cell cycle regulation, differentiation, growth, and aging [2,3]. LS was firstly reported by Zeisler and Becker in 1936, in a 24-year-old woman presenting with multiple lentigines, increasing in number from birth to puberty [2). A few decades later, Gorlin et al. reviewed this disorder and introduced the LEOPARD acronym supporting the concept of a more generalized condition [4].
The acronym stands for: multiple Lentigines, Electrocardiographic conduction abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, growth Retardation and sensorineural Deafness [4]. Patients with LS do not usually present with all of these classical clinical features. However, multiple lentigines, are considered to be the most striking sign, even if they may be lacking in the early childhood. They are flat, black-brown macules, mainly affecting the face, the neck and the upper chest but can appear all over the body, invariably sparing the mucosa [2,5]. Associated café-au-lait spots have also been reported in some patients with LS [2,3].
Herein, we describe a family with members exhibiting multiple lentigines and café-au-lait spots.

PATIENTS AND METHODS

The family members were from two generations: father and his two children. The father was never diagnosed with LS before and had no medical record. Clinical data for the two children were col-

lected from their medical records. DNA samples of the two parents and both children were taken after consent. The molecular analysis of the patients' DNA was performed by next-generation sequencing (NGS).

CASES

Case 1 : The father was a 51-year-old male with no medical history of deafness, cryptorchidism or pulmonary stenosis. The lentigines were noticed in the early childhood and had progressively increased until puberty. They were taken for freckles and birth marks. By history, his elder brother appeared to be having the same skin lesions. Neither the brother nor the other siblings were seen or examined.
The patient was 160 cm tall and weighed 70 kg, with no dysmorphic features. There were multiple dark-brown macules, mainly on the face, with two café-au-lait spots on the trunk.

Case 2 : The 13-year-old son had a medical history of bilateral cryptorchidism for which he underwent orchodopexy at the age of 14 months. He was also operated for umbilical hernia at the age of 8 years. He had mild learning difficulties resulting in a poor school performance. On physical examination, a short stature (132 cm) below 25th percentile and dysmorphic features were noticed: hypertelorism and large low-set ears. He presented with multiple dark-brown lentigines mainly on the face 1 to 4 mm in size and café-au-lait spots with the largest one on the right ankle measuring 2 cm (Figure 1). The lentigines were firstly noticed at the age of 3 years, and had multiplied and become darker by time. Neither chest deformation nor skeletal anomalies were noticed.
The clinical evaluation of LEOPARD syndrome was based on an electrocardiogram (ECG), dynamic ECG (holter monitor), an echocardiogram, an audiometry and an MRI for the brain. They were all normal. An ophthalmologic examination was performed and showed myopia. Blood smear revealed bicytopenia: leucopenia at 3000/µL and thrombocytopenia at 120.109/L. A bone marrow biopsy was performed and showed no sign of myelodysplasia.

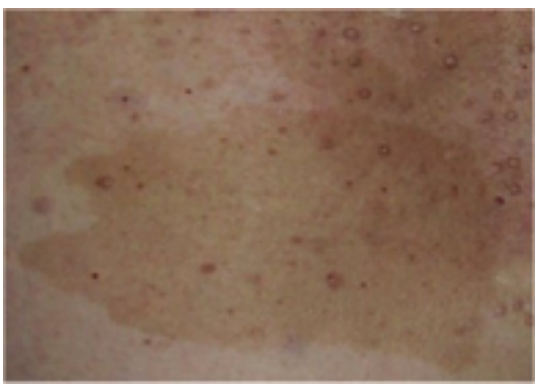

Figure 1 : Typical lentigines and café-au-lait spots on the right ankle (case 2)

Case 3 : The 8-year-old daughter underwent cochlear implantation on the left side at the age of 3 years, for congenital sensorineural hearing loss. The parents reported a gross motor and speech delay with real improvement after speech and language therapy. On general examination, she had low-set ears and ocular hypertelorism like her brother, added to dental implantation abnormalities. She was 112 cm tall (<3rd percentile). She presented with multiple lentigines on the neck, trunk, and lower extremities, and multiple café-au-lait spots, with the largest one measuring 1cm located in the left iliac fossa. The first lentigo had appeared at the age of 5 years. As for the café-au-lait spots, they had been noticed since birth. That was the case for her elder brother too. The electrocardiogram, dynamic ECG (holter monitor), echocardiogram and brain MRI showed no abnormalities. The ophthalmologic examination revealed bilateral amblyopia. Abdominal ultrasound showed no kidney or ovary abnormalities.
Both children were a product of a nonconsanguineous marriage, uneventful pregnancies and deliveries with no perinatal complications.

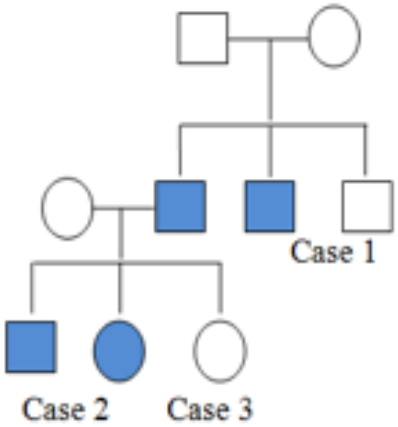

Figure 2 : Family pedigree. Filled symbols indicate the affected individuals based on clinical grounds.

Genetic investigations:

Molecular analysis of genomic DNA of the parents and their two children by NGS revealed a heterozygous missence mutation in PTPN11, (c.836A>G;p.Y279C) in the 7th exon for the three clinically affected patients, which is one of the most recurrent mutations for LS. The mother carried the wild-type allele of the identified PTPN11 variant.

Table 1 : Clinical manifestations of the three cases

Manifestations		Case1	Case2	Case3
L	Multiple Lentigines	+	+	+
E	ECG abnormalities	-	-	-
O	Ocular hypertelorism	-	+	+
P	Pulmonary stenosis	-	-	-
A	Abnormal genatalia	-	+	-
R	Growth Retardation	+	+	+
D	Sensorineural Deafness	-	-	+
Skin	Café-au-lait spots	+	+	+
Ears	Low-set ears	-	+	+
Hematological	Thrombocytopenia	-	+	-
	Leukopenia	-	-	-
Others	Speech delay	-	-	+
	Gross motor delay	-	-	+
	Dental implantation abnormalities	-	-	+
	Umbilical hernia	-	+	-
	Myopia	-	+	+

DISCUSSION

We presented the case of a family showing the same point mutation with variable expressivity.
Extremely variable phenotypes have been reported in relation to LS. Voron et al. categorized the LS manifestations as follows: 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. They established minimal diagnostic criteria for LS: multiple lentigines with at least two of the other major features or, if lentigines are absent, three of the other cardinal features and a first-degree relative with LS [6]. All our patients meet these criteria.

Skin lesions

Both lentigines and café-au-lait spots were noticed in our reported cases. Lentigines are the most prominent manifestation of LS, present in more than 90% of the patients [5]. They develop classically during childhood, increasing in number and darkening in color with age. Café-au-lait spots can be also observed, alone or in association with lentigines, in up to 70–80% of the patients [5].
Cases 2 and 3 had café-au-lait spots were noticed at birth. The diagnosis was not made until the age of 3 years for the boy (case2) and 5 years for the girl (case 3) when lentigines began to appear. In the literature review, the mean age at report was also around infancy and during childhood [2,5].

Dysmorphic features

Dysmorphic features can occur or be only mildly expressed in newborns and infants [2], explaining the absence of these clinic signs in the father (case 1). Both children (case 2 and 3) displayed hypertelorism and low-set ears. Hypertelorism is virtually present in all cases [5] while low set-ears are less frequently reported.

Cardiovascular system

Fortunately, no hypertrophic cardiomyopathy (HCM) was noted in our patients even though it is considered to be the most frequent cardiac anomaly observed in up to 80% of cases [2,5]. Careful cardiological assessment is required as HCM, electrocardiographic and ultrasound abnormalities such as ventricular hypertrophy, right atrial enlargement, right bundle branch,
paroxysmal atrial tachycardia, atrial fibrillation, prolonged PR interval, left anterior and posterior hemiblocks, bundle branch block or complete atrioventricular block, may occur later [5,7].

Growth retardation and skeletal anomalies

Only 25% of affected individuals express growth retardation [5,6]. Short stature was noticed in all our cases.Meanwhile, none of our three patients showed skeletal anomalies such as broad chest, pectus carinatum or excavatum. Thorax anomalies are found in up to 75% [2]. These signs are more common in newborns, and may have disappeared in time.

Neurological abnormalities

Visual abilities are commonly impaired [5], in agreement with our results, requiring regular follow up and correcting of the refractive ametropia.
Mild learning difficulties are reported in about 30% of the cases [2]. Poor school performances and speech delay were found in both children (case 2 and 3), while their father was not evaluated. Both of them (case 2 and 3) underwent brain imaging as suggested for LS patients displaying neurologic abnormalities [5].

Sensorineural hearing loss

Sensorineural deafness occurs in about 15–25% of patients [2,5]. Although most cases are diagnosed at birth or during childhood, hearing tests are required to adulthood for the brother since deafness may occur later in life [2,5].

Genitourinary abnormalities

Bilateral cryptorchidism, reported in case 2, occurs in about half male cases. His sister may show late onset of puberty as well as hypoplastic ovary.

Other clinical manifestations

Dental anomalies might be expected in some cases of LS. They are due to damage of neural crest cells that participate in the formation of the teeth [8]. Case reports showed variability in the dental phenotype. High-arched palate, primary maxillary central incisor caries, missing primary mandibular left lateral incisor and increased overjet have been described [8]. Dental abnormal implantation was reported in our study (case3).
Case 2 displayed umbilical hernia, which was rarely reported in literature reviews [5].

Tumors

Mutations in RAS genes can lead to overactive signaling inside the cell, ultimately leading to cancer, particularly acute lymphoblastic leukemia, acute myeloid leukemia and rhabdomyosarcoma [10]. Thus, LS patients with PTPN11 mutations should be closely monitored for malignancy, particularly du-

ring their childhood. Fortunately, for case 2, bone marrow biopsy showed no sign of myelodysplasia. For the other two cases, blood smear was normal.

Mutations

The identification of the causative genes that underlie LS has facilitated molecular diagnosis of these disorders, enabling the evaluation of genotype–phenotype relationships. To date, 12 missense PTPN11 related to LS have been identified: Tyr-279Cys/Ser, Ala461Thr/Ser, Gly464Ala, Thr468Met/Pro, Arg498Leu/Trp, Gln506Pro, and Gln510- Glu/Pro [3,5,11]. The cases we reported carry the c.836A > G missense mutation in exon 7 (p.Tyr279Cys), which is one of two most frequent mutations that account for more than 60% of cases [2,4,5]. Short stature, characteristic facies, deafness and developmental delay are classically associated with the p.Tyr279Cys mutation [4,5,11]. However, long-term cardiac evaluation should be considered in our cases since HCM rarely lacks.

In **CONCLUSION,** familial lentigines and café-au-lait spots must trigger a search for LEOPARD syndrome. Although all family members included in our study had the same p.Tyr279Cys mutation in PTPN11, clinical features were not identical, thus showing the phenotypical diversity of the point mutation.

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