Two cases of LEOPARD syndrome – RAF1 mutations firstly described in children

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LEOPARD syndrome 2 (LS-2) (OMIM #611554) is a rare, dominantly inherited genetic disorder affecting multiple organ systems. We report two unrelated females of different ages whose phenotype fits best in the category of LEOPARD syndrome, both with proven mutations in the RAF1 gene not previously reported in pediatric patients. In our 10-year-old patient, who was negative in the PTPN11 gene analysis but involving the RAF1 gene in a complementary analysis, the sequence variant Ser257Leu (770C>T, exon 7) was detected, which previously had been reported in only one 35-year-old woman with LS. The second patient was a two-year-old female infant with Ser259Leu mutation in the same gene, described in several patients with Noonan syndrome (NS) but not in LS patients of any age. The first girl had ventricular and supraventricular extrasystoles, and the second had episodes of paroxysmal supraventricular tachycardia. Echocardiographic examination revealed biventricular obstructive hypertrophic cardiomyopathy in both patients.

Key words: LEOPARD syndrome, RAF1, p.Ser257Leu mutation, p.Ser259Leu mutation, obstructive hypertrophic cardiomyopathy.

LEOPARD syndrome (LS, OMIM #151100; LS-2, OMIM #611554) is an acronym for the manifestations of the syndrome as listed by Gorlin et al.[2]: Lentigines, Electrocardiographic anomalies, Ocular hypertelorism/Obstructive cardiomyopathy, Pulmonic stenosis, Abnormal genitalia, Retardation of growth, and sensorineural Deafness[1,2].

LEOPARD syndrome (LS) shares many features with Noonan syndrome (NS), in which lentigines and deafness are usually not present. Digilio et al.[4] showed that NS and LS are allelic disorders.

To date, mutations associated with LS have been identified in exons 7, 12 and 13 of PTPN11, exons 7, 14 and 17 of the RAF1 gene, and exon 6 of the BRAF gene[3-6]. The genes that cause LS and related disorders encode components of the RAS-MAPK pathway[1,4,6,7].

LEOPARD syndrome (LS) may be sporadic or inherited as an autosomal dominant fully penetrant trait with variable expressivity. About 200 patients have been reported worldwide, but the real incidence is still unknown[1]. A few cases of LS have been reported from the Balkan region, particularly Turkey and Greece, but none from Serbia[8,9].

The aim of these reports is to give a detailed phenotypic description of two cases of LS with RAF1 mutations firstly identified in two unrelated female children of different ages.

Case Reports

The patients were diagnosed with LS at the Mother and Child Healthcare Institute of Serbia, Belgrade, based upon the clinical features and the diagnostic criteria proposed by Voron et al.[10].

Case 1

A 10-year-old girl was born as the second child of nonconsanguineous parents. There were no concerns regarding her two siblings, and there was no other relevant family history. She was born at the 36th week of gestation with a birth weight of 2650 g (P_{55} centile for 36th week) after an uncomplicated pregnancy. The female neonate experienced progressive onset of severe heart failure and had complete symptoms recovery. Characteristic facial features and hypotonia were noted in the neonatal period.

At the age of three months, biventricular hypertrophic cardiomyopathy (HCM) with concentric hypertrophy of the left ventricle was diagnosed by echocardiography. At the age of three years, she had episodes of supraventricular tachycardias, and antiarrhythmic therapy was started (beta-blockers). The presence of ventricular extrasystole (VES), supraventricular extrasystole (SVES), as well as incomplete right bundle branch block (IRBB) was confirmed by 24-hour Holter ECG monitoring. Later, echocardiographic examination showed HCM with significant and asymmetric right and left ventricular outflow tract obstruction (RVOTO and LVOTO), assessed as moderate. There was mitral, tricuspid and pulmonary valve regurgitation.

At the age of 10, her height was 122 cm (3.5 cm below P_3). She had facial anomalies during childhood, such as palpebral ptosis and prominent eyes, as well as short neck, broad chest, pectus excavatum, and scoliosis (Fig. 1). She developed multiple pigmented nevi and

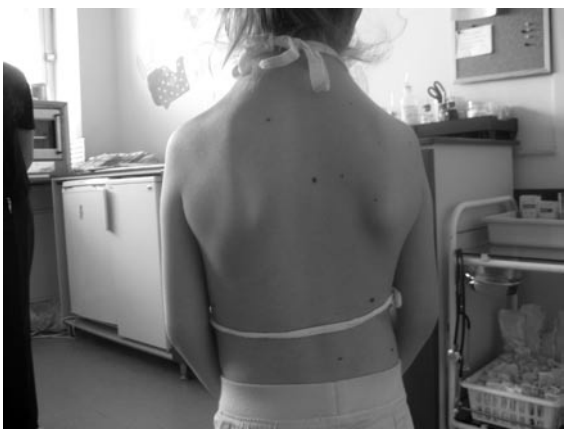

Figure 1: ***Case 1:*** Kyphoscoliosis and lentigines on the back.

Figure 2: Facial appearance of Case 1 with p.Ser257Leu mutation, showing phenotypic features of LEOPARD syndrome.

lentigines at the age of four. The largest was under the left orbit, 4 cm in diameter and hairy in the center (Fig. 2). There were no pubertal signs. Ultrasonographic examination did not show hypoplastic ovarian changes. At the most recent developmental assessment at the age of 10, she had IQ_{total} 80 (IQ_{verbal} 80; $IQ_{motoric}$ 79), characterized as mild mental retardation. Audiometric assessment did not show any sensorineural changes.

Case 2

The second patient, a female infant aged 2 and 6/12 years, was the first child of healthy unrelated parents. She was delivered at the 42nd week of gestation by cesarean section. She did not cry immediately, so she was intubated and reanimated for 30 minutes. Her birth weight and length were 3350 g (P_{75} for 42nd week) and 49 cm (P_{25} for 42nd week), respectively. Interatrial septal defect, hypotonia and convulsions were diagnosed in the neonatal period, as well as characteristic facial features, and high and wide forehead with frontal protuberance.

Echocardiographic examination revealed biventricular obstructive HCM at the age of three months. Later, echocardiographic examination showed asymmetric HCM with significant RVOTO and mitral regurgitation. At the age of two years, she had episodes of supraventricular tachycardias, so antiarrhythmic therapy was started (beta-blockers). The presence of SVES was confirmed by 24-hour Holter ECG monitoring.

At the age of 2 and 6/12 years, her height was 78.5 cm (7.5 cm below P_5). The cranium was dolichocephalic (Fig. 3). The face was flattened. She had palpebral ptosis, especially on the right side. She had scoliosis, thoracal kyphosis and lumbar lordosis. She had several pigmented nevi and lentigines with maximal diameter of 0.5 cm on the supraclavicular left and para-cubital left regions and one café-au-lait of 2 cm in diameter on the right humeral region (Fig. 4).

Audiometric testing did not show any sensorineural changes. Ultrasonogram of the central nervous system showed cysts of 4 mm in the right plexus choroideus.

Mutational Analysis

Both patients had normal 46,XX karyotype. The whole PTPN11 gene was directly sequenced from genomic DNA of the patient, and no alterations were identified. Then, the coding sequences of RAF1 exons 7, 14 and 17 were polymerase chain reaction (PCR)-amplified, and direct sequencing was performed on the PCR products as previously described[3,5]. A heterozygous RAF1 mutation was detected by direct sequencing of exon 7; the first patient had p.Ser257Leu mutation and the second had p.Ser259Leu mutation.

Discussion

We presented herein two unrelated females of different ages whose phenotypes fit best in the category of LS-2, both with proven mutations in the RAF1 gene not previously reported in pediatric patients. In our patients, the clinical data confirmed the diagnosis of LS, and mutations explained the phenotype encountered.

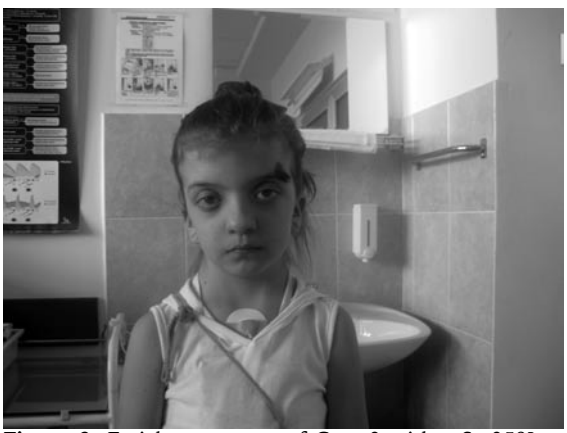

Figure 3: Facial appearance of Case 2 with p.Ser259Leu mutation, showing phenotypic features of LEOPARD syndrome.

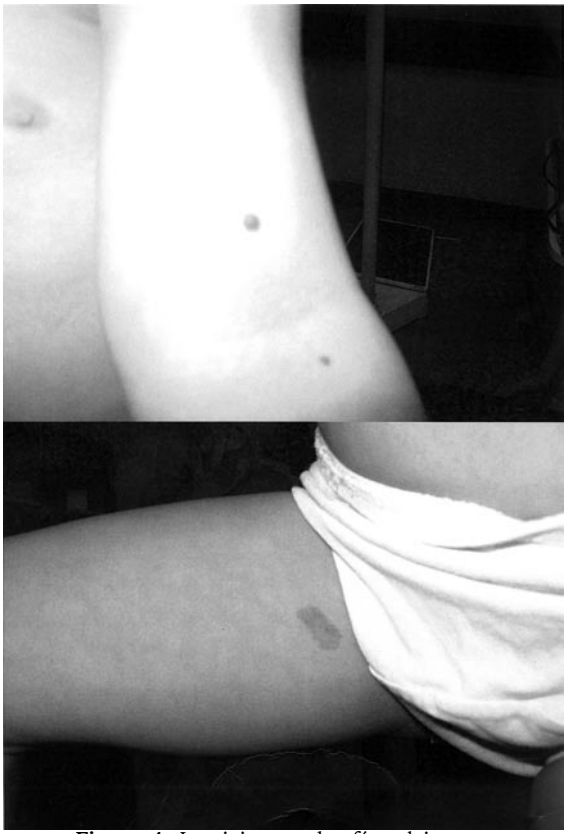

Figure 4: Lentigines and café-au-lait spot.

In the first patient, a 10-year-old girl, the sequence variant Ser257Leu (770C>T, exon 7) in the RAF1 gene was detected, and was not previously reported in pediatric patients (only in one 35-year-old woman with LS)[3]. The second patient, a two-year-old female infant, had the Ser259Leu mutation in the same gene, which had been described in several patients with NS, but not in LS patients of any age. Clinical diagnosis of LS is generally difficult to establish in the first months of life because the distinctive lentigines are not generally present at birth and develop later during childhood. Lentigines classically develop at 4-5 years[1,3]. Some individuals with LS do not exhibit lentigines.

The diagnosis of LS in the first months of age can be clinically suspected in patients having three main features: characteristic facial marks (100%), HCM (87%), and café-au-lait spots (75%)[4].

PTPN11 mutations have been identified in approximately 50% of patients with NS and in 90% of LS cases[1]. Pandit et al.[3] reported RAF1 gene (3p25) mutations in 3% and 30% of patients with NS and LS, respectively, without known mutations. The RAF1 gene is highly regulated with numerous serine and threonine residues that can be phosphorylated, resulting in activation or inactivation of the RAS/MAPK cascade via RAF1 MEK kinase activity[5,9-11].

Kontaridis et al.[11] concluded that since LS and NS have different pathogenesis (loss-of-function in LS, gain-of-function in NS), they should be distinguished based on mutation analysis. Furthermore, it is still unknown how mutations that have opposite biochemical effects can lead to similar phenotypes. Nevertheless, mutation analysis is a valuable tool for the differential diagnosis. On the other hand, proper clinical evaluation can be the clue in some patients, like in Case 2.

Both our patients had a severe form of biventricular obstructive left ventricular hypertrophy (LVH) consistent with previous observations[12]. HCM can be congenital, but is typically manifested during later infancy and may be associated with significant LVOTO in up to 40% of cases[1,3,5,7].

Congenital heart diseases are often the first clinical problem leading to medical attention[4].

Approximately 85% of affected individuals have heart defects, including HCM and pulmonary valve stenosis[12,13]. All reported patients with RAF1 mutations had HCM. Compared to children with HCM, those with LVH in the setting of LS show more ventricular hypertrophy and diastolic dysfunction due to abnormal relaxation and reduced compliance and RVOTO[3,5,13].

Mitral valve prolapse, present in the first patient, has been found in up to 42% of cases. Less frequent heart defects are atrial and atrioventricular septal defects, coronary artery abnormalities and apical aneurysm[1,3]. Atrial septal defect was noticed in the second patient.

Electrocardiogram abnormalities occur in about 75% of patients[1]. The first female had VES and SVES, and the second had episodes of paroxysmal supraventricular tachycardia (PSVT).

Limongelli et al.[13] suggested that patients without PTPN11 mutations showed a significantly higher frequency of family history of sudden death, increased left atrial dimensions, episodes of supraventricular bradyarrhythmias, supraventricular tachycardias, fibrillation, nonsustained ventricular tachycardias, and other adverse arrhythmic and nonarrhythmic events[12,13].

Birth weight is normal, like in our cases, or above the average in one-third of the newborns[1].

Postnatal growth retardation resulting in short stature occurs in 25% of affected persons below the 3rd centile in height and 85% of adults below the 25th centile[1], like in our patients.

Noonan et al.[14] reported that adult height was not connected with the presence or severity of cardiac disease, and none of the adults achieving a normal height was treated with growth hormone. The increase in bone age was equal or slightly greater than the overall increase in height, so it is suggested that growth hormone therapy could actually decrease the adult height[14]. In general, LEOPARD patients are taller than the patients with PTPN11 NS[14].

Because of the high incidence of HCM in LS, many are reluctant to use growth hormone, although the possible adverse effects of growth hormone on HCM are still not fully recognized. Delayed puberty and hypoplastic ovary have been reported in females, but it was not seen in Case 1.

Sensorineural deafness occurs in about 15-25% of patients[1]. Most cases are diagnosed at birth or during childhood, but deafness may also develop later[1]. Neither of our patients had deafness or any sensorineural changes. Mental retardation, typically mild, is found in approximately 30% of individuals with LS[1,4]. Our first patient had mild mental retardation.

Early diagnosis of the diseases is useful for the surveillance of the specific medical problems associated with LS and for adequate genetic counseling to the families[4]. It is unclear whether the genotype may influence the clinical course in the patients with LS.

With the exception of only ventricular hypertrophy and arrhythmias, patients with

LS do not require special medical care, and long-term prognosis is favorable[1,2,11].

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