Molecular Genetics of LEOPARD Syndrome

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Article Contents

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LEOPARD syndrome (LS) is an acronym for the cardinal features lentigines, electrocardiogram conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth and sensorineural deafness. However, other disorders, such as hypertrophic cardiomyopathy, occur frequently and represent a potentially life-threatening problem. PTPN11 mutations, located on chromosome 12q24, are observed in up to 90% of patients with LS. Meanwhile, mutations in the RAF1 gene on chromosome 3p25.2 and mutations in the BRAF gene on chromosome 7q34 occur in 5% of the cases. Eleven different missense PTPN11 mutations, characterised by a decrease in physiological activity of the mutated protein (Tyr279Cys/Ser, Ala461Thr, Gly464Ala, Thr468Met/Pro, Arg498Trp/Leu, Gln506Pro and Gln510Glu/Pro) have been reported, two of which (Tyr279Cys and Thr468Met) occur in approximately 65% of the cases.

Introduction

A class of human genetic syndromes has emerged that are caused by germline mutations in genes which encode components of the RAS/mitogen-activated protein kinase (MAPK) pathway. RAS proteins (HRAS, NRAS and KRAS) are small guanosine-binding proteins, which act as signal switch molecules involved in transmitting extracellular inputs downstream into the cell and its name 'RAS' is an abbreviation of 'Rat sarcoma', reflecting the way the first members of the protein family were discovered. Meanwhile, MAPK comprises a family of ubiquitous proline-directed, protein–serine/threonine kinases, which participate in signal transduction pathways that control intracellular events including acute responses to hormones and major developmental changes in organisms (Pearson *et al.*, 2001). The RAS/MAPK pathway was initially studied in relation to oncogenesis, as it is dysregulated in 20–30% of somatic tumours (Bos, 1989; Parikh *et al.*, 2007). However, unlike somatic mutations in the RAS pathway which have a very high malignant potential, germline mutations lead to developmental abnormalities that are often poorly clinically differentiated. These disorders, the so-called neurocardiofaciocutaneous syndromes or RASopathies, share many clinical features and a common pathophysiology and are characterised by facial dysmorphism, a wide spectrum of cardiac disease, postnatal reduced growth, ectodermal and skeletal defects, variable cognitive deficits and, in some cases, a predisposition to cancer (Digilio *et al.*, 2011; Tartaglia *et al.*, 2010).

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RAS-MAPK Signal Transduction Pathway

The RAS-MAPK signal transduction pathway is activated through growth factors binding to receptor tyrosine

kinases, G-protein-coupled receptors, cytokine receptors and extracellular matrix receptors. Receptor autophosphorylation favours its interaction with the adaptor growth factor receptor-bound protein 2, which in turn binds to SOS1 protein and increases the RAS nucleotide exchange rate of guanosine diphosphate for guanosine triphosphate (GTP), resulting in an increased RAS in the active GTP-bound form. In addition, SHP2 is an allosteric phosphatase essential for growth factor-mediated Ras activation (Yu *et al*., 1998, 2013). Activated RAS binds and leads to the activation of BRAF and RAF1 (also named CRAF), both belonging to the group of MAP3K proteins. Once activated, BRAF and RAF1 can phosphorylate and activate the dual specificity protein kinases MEK1 and MEK2 (MAP2K), which in turn phosphorylate and activate the serine/threonine-specific protein kinases ERK1 and ERK2 (MAP K). ERK1/2 are the ultimate effectors and exert their function on a large number of downstream molecules, both nuclear and cytosolic (Aoki *et al*., 2008; Yoon and Seger, 2006; **Figure 1**). This addition of phosphate groups to a neighbouring protein in the RAS-MAPK signal transduction pathway acts as an 'on' or 'off' switch. When one of the proteins in the pathway is mutated, it can be stuck in the 'on' or 'off' position, which is a necessary step in the development of many cancers or phenotypically similar diseases such as Noonan syndrome (NS, OMIM #163950), Costello syndrome (CS, OMIM #218040), cardiofaciocutaneous syndrome (CFC, OMIM #115150) or LEOPARD syndrome (LS, OMIM #151100). **See also**: Cardiofaciocutaneous (CFC) Syndrome; Noonan Syndrome

With perturbations of the MAPK signalling pathway established as central to RASopathy disorders, several candidate genes along this pathway have been identified in humans with RASopathy disease phenotypes, including mutations in RAS, SOS1, BRAF, RAF1, MEK1, MEK2 or PTPN11 (protein–tyrosine phosphatase, nonreceptor type 11) genes (Tidyman and Rauen, 2009). However, mutations affecting this pathway can also occur in a mosaic state, resulting in congenital syndromes often distinct from those generated by the corresponding germline mutations (Hafner and Groesser, 2013).

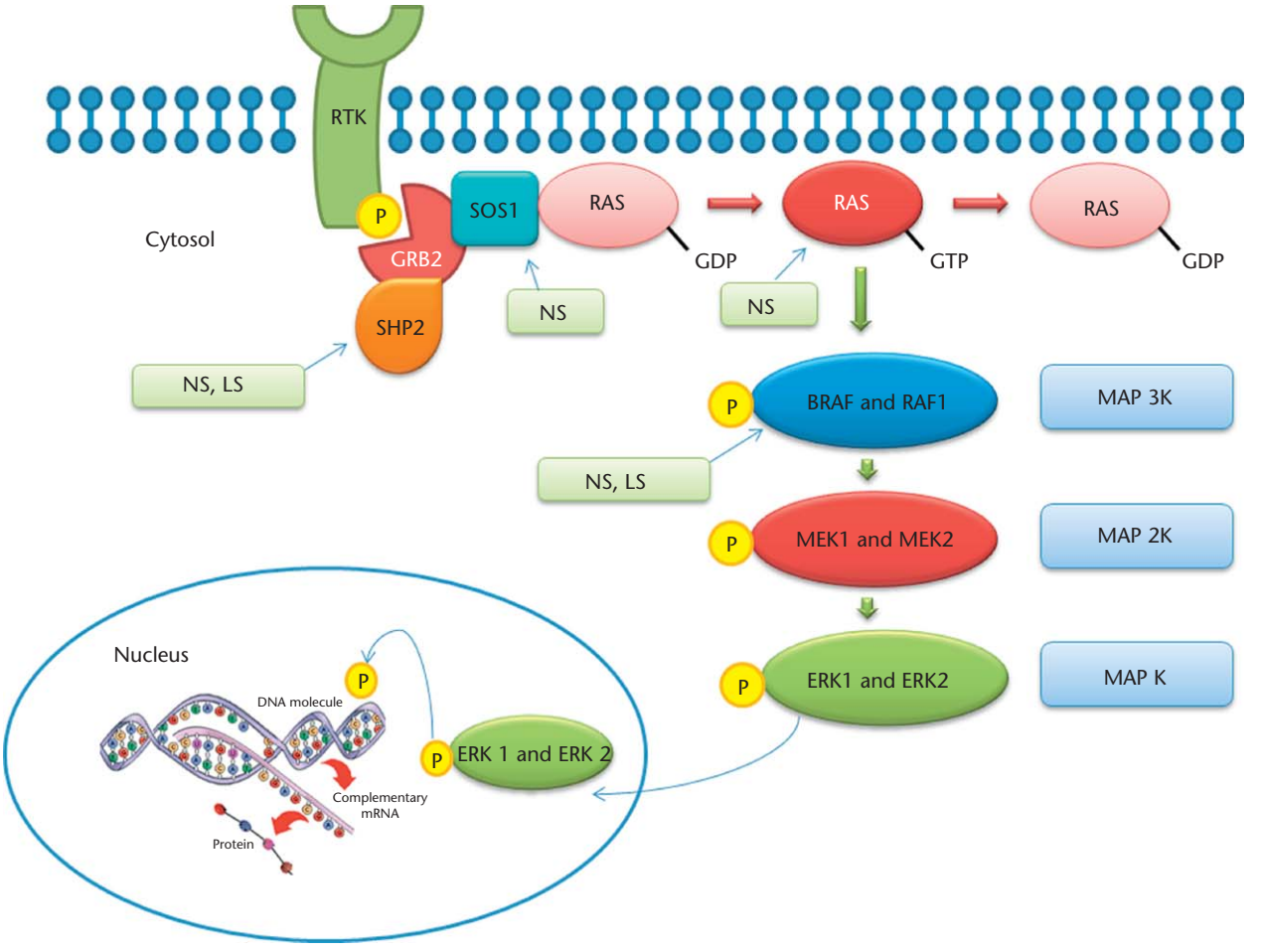


Figure 1 Schematic diagram showing the RAS-MAPK signal transduction pathway and proteins that are affected in both syndromes (LS, Leopard syndrome and NS, Noonan syndrome).

PTPN11 Gene

PTPN11 gene encodes tyrosine–protein phosphatase nonreceptor type 11, also known as SHP-2 (**Figure 2**). SHP2 protein contains a tandem arrangement of two SH2 domains, at the *N*-terminus (*N*-SH2 and *C*-SH2), a single catalytic domain (PTP) and a *C*-terminal tail containing two tyrosyl phosphorylation sites and a proline-rich stretch whose function is not well characterised (Tartaglia *et al*., 2006). SHP2 has inactive and active conformations that are regulated through a molecular switching mechanism. In the inactive state, the backside of the *N*-SH2 domain forms a loop and is wedged into the PTP domain, blocking the catalytic site. When SHP2 binds phosphotyrosyl-containing proteins at separate sites on the *N*- and *C*-SH2 domains, a conformational change releases the *N*-SH2 domain from the PTP domain, which makes the PTP catalytic cleft available. Mutations alter residues at or close to the *N*-SH2/PTP interacting surfaces, which are involved in switching between active and inactive conformations of the protein, and participating in catalysis.

PTPN11 mutations, located on chromosome 12q24, are observed in up to 90% of the patients with LS (Lauriol and Kontaridis, 2011). However, mutations in the RAF1 gene on chromosome 3p25.2 and mutations in the BRAF gene on chromosome 7q34 can also be found in a small percentage (5%) of cases (Pandit *et al*., 2007). Eleven different loss-of-function or dominant-negative missense PTPN11 mutations, characterised by a decrease in physiological activity of the mutated protein (Tyr279Cys/Ser, Ala461Thr, Gly464Ala, Thr468Met/Pro, Arg498Trp/Leu, Gln506Pro and Gln510Glu/Pro) have been reported, two of which (Tyr279Cys and Thr468Met) occur in approximately 65% of the cases (Sarkozy *et al*., 2008, 2004; **Table 1**).

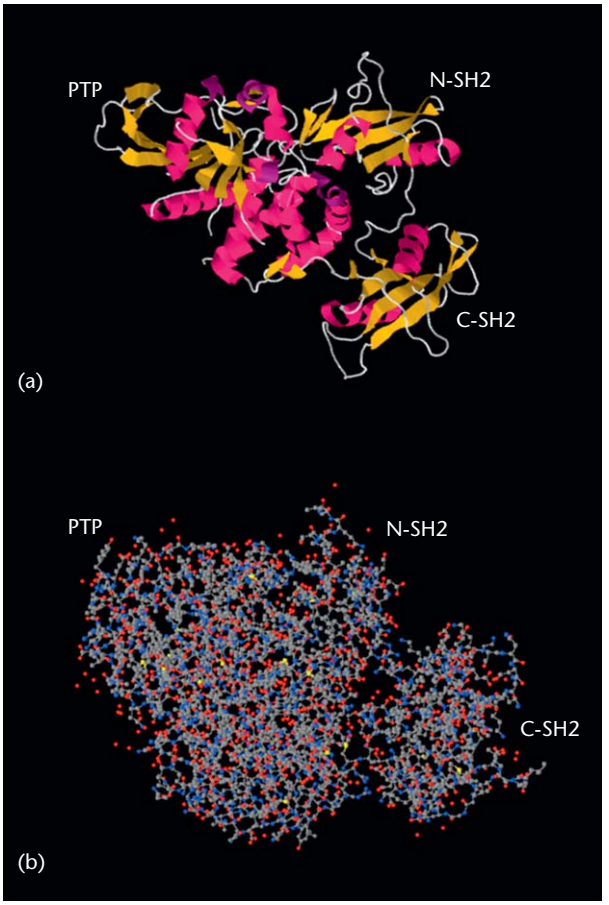


Figure 2 The wild-type tyrosine–protein phosphatase nonreceptor type 11 (SHP2) protein. Diagram showing secondary structure and organisation of the domains (a) and 3D image (b) of SHP2 (structures in (a) and (b) are in similar orientations). In the protein tyrosine phosphatases (PTP) family, a subgroup of cytoplasmic PTPs characterised by containing two Src homology 2 (SH2), NH_2-terminal domains and a C-terminal protein-tyrosine phosphatase domain, are referred to as SHP. Note that the peptide-binding sites of both SH2 domains are exposed on the molecular surface. A distinct surface of the *N*-SH2 domain occupies the active site of the PTP domain (autoinhibited closed configuration). Image modified from the RCSB PDB (www.pdb.org) of PDB ID 10.2210 (Yu *et al*., 2013).

RAF1 Gene

RAF1 gene that encodes RAF1 protein comprises 17 exons and has three conserved regions (CR). CR1, exons 2–5, contains a RAS-binding domain and a cysteine-rich domain, CR2 lies in exon 7 and CR3, which spans exons 10–17, contains the kinase domain and its regulatory element, the activation segment. The gene is highly regulated

Table 1 Human genome organisation (HUGO) approved gene designations for PTPN11, RAF1 and BRAF genes in LEOPARD syndrome

PTPN11		
NM_002834.3[a]	NP_002825.3[b]	dbSNP ID[c]
c.836A>G	p.Tyr279Cys	rs121918456
c.836A>C	p.Tyr279Ser	
c.1381G>A	p.Ala461Thr	rs121918468
c.1391G>C	p.Gly464Ala	rs121918469
c.1402A>C	p.Thr468Pro	
c.1403C>T	p.Thr468Met	rs121918457
c.1492C>T	p.Arg498Leu	
c.1493G>T	p.Arg498Trp	
c.1517A>C	p.Gln506Pro	
c.1527A>C	p.Gln510Pro	rs121918470
c.1528C>G	p.Gln510Glu	
RAF1		
NM_002880.2[a]	NP_002871.1[b]	dbSNP ID
c.770C>T	p.Ser257Leu	rs80338796
c.1837C>G	p.Leu613Val	rs80338797
BRAF		
NM_004333.4[a]	NP_004324.2[b]	dbSNP ID
c.721A>C	p.Thr241Pro	
c.735A>T	p.Leu245Phe	

[a]Nucleotide reference sequence.
[b]Protein reference sequence.
[c]Single-nucleotide polymorphism database identity.

with numerous serine and threonine residues that can be phosphorylated, resulting in activation or inactivation. RAF1 pathologic allelic variants causing LS are Ser257Leu and Leu613Val. However, and in contrast to what happens with the PTPN11 mutations, gain-of-function mutations of RAF1 gene have been identified in a small percentage of LS patients (Pandit *et al*., 2007). The Ser257Leu variant alters the CR2 domain and causes greater kinase activity than wild-type protein, both basally and after stimulation (Pandit *et al*., 2007). Meanwhile, Leu613Val mutation alters the *C*-terminal portion of RAF1 (coding exon 16) causing greater kinase activity than wild-type protein, both basally and after epidermal growth factor stimulation (Gelb and Tartaglia, 1993–2013; **Table 1**).

BRAF Gene

However, the BRAF gene is ubiquitously expressed and encodes the BRAF protein which plays a role in regulating the MAPK/ERK signalling pathway, which affects cell division, differentiation and secretion. Human BRAF gene comprises 18 coding exons and pathologic allelic BRAF variants causing LS are Thr241Pro and Leu245Phe. Thr241Pro BRAF mutation enhances RAS signalling through increased activation of MEK and ERK kinases (Sarkozy *et al*., 2009; **Table 1**).

Epidemiology and Inheritance

Although no epidemiologic data are available, the syndrome seems to be infrequent. Nonetheless, within the group of the so-called neurocardiofaciocutaneous syndromes or RASopathies, LS is probably the second most common disorder after NS which has an estimated incidence between 1:1000 and 1:2500 live births (van der Burgt, 2007).

LS may be sporadic (*de-novo* mutation) or inherited as an autosomal dominant trait. This means that the patient can be the first affected person in his/her family or that the patient has a 50% chance to inherit the mutated gene, respectively. However, as expressivity is indeed variable, not all individuals who inherit the mutation go on to develop the disease. Genetic counselling is an important part of medical care because it helps families and patients to understand and cope with the diagnosis of their genetic condition and its implications. Providing adequate information, including discussion of potential risks to offspring and reproductive options, will ensure them to make informed medical and personal decisions.

Clinical Features

LS is an acronym for the cardinal features lentigines, electrocardiogram (ECG) conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth and sensorineural deafness. However, other disorders are also frequent.

Facies

The facial dysmorphism can occur or be only mildly expressed in the newborns and infants. Hypertelorism is virtually present in all cases whereas flat nasal bridge and dysmorphic ears occur in approximately 87% of the patients (**Figure 3a**). Similarly, other facial features such as palpebral ptosis, low-set ears, deep nasolabial folds and premature skin wrinkling are frequently seen.

Skin

Lentigines are the most prominent manifestation of LS and are found in more than 90% of the patients even though an absence of the feature does not exclude the diagnosis of the syndrome. Multiple lentigines present as dispersed flat, black–brown macules, mostly on the face, neck and upper part of the trunk with sparing of the mucosa (**Figure 3**). Although lentigines may be present at birth they usually appear during childhood becoming more numerous and darker with age, independentof sun exposure. Café-au-lait spots are also observed, alone or in association with

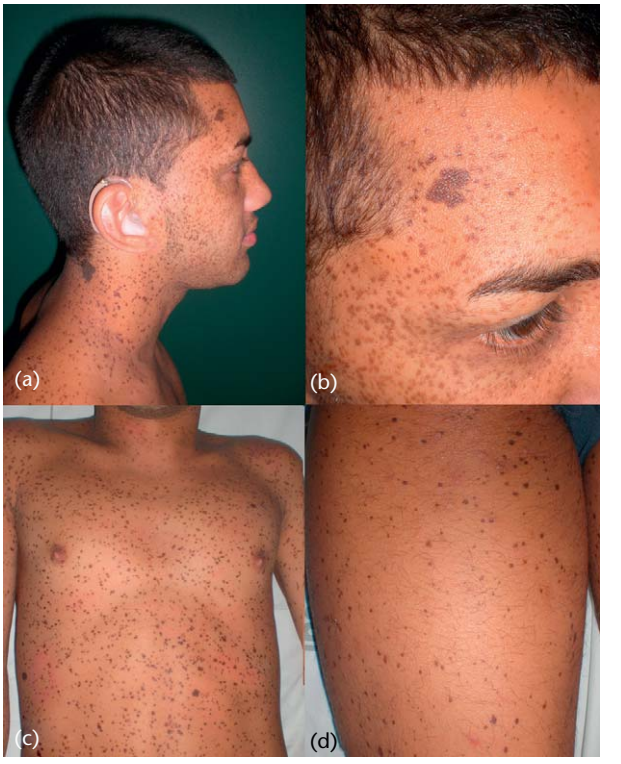


Figure 3 A LEOPARD syndrome patient with hearing aids due to sensorineural deafness (a); numerous lentigines on the skin of the face (b), chest (c) and legs (d).

lentigines, in up to 70–80% of the patients (Digilio *et al*., 2006) and are similar to those found in neurofibromatosis type1 and Legius syndrome. **See also**: Legius Syndrome and SPRED1; Neurofibromatosis Type I (NF1)

Cardiac Defects

In all, 85% of patients with LS have heart defects and although it is not included in the acronym, hypertrophic cardiomyopathy (HCM) is the most frequent anomaly observed, representing a potentially life-threatening problem. HCM, which is generally asymmetric and progressive and commonly involves the intraventricular septum, is detected in up to 80% of the patients with a cardiac defect and may be associated with significant left ventricular outflow tract obstruction in up to 40% of the cases (Limongelli *et al*., 2007; Sarkozy *et al*., 2008; Martínez-Quintana and Rodríguez-González, 2011). In fact, left ventricular hypertrophy is sometimes visible on the chest X-ray (**Figure 4a**) and on the 12-lead ECG (**Figure 4b**) having both methods a good correlation with ventricular hypertrophy. However, the highest sensitivity for the evaluation of heart growth is obtained with transthoracic echocardiogram (**Figure 5a**) and magnetic resonance imaging (**Figure 5b**).

Although, without an evidence-based diagnosis, treatment algorithms are similar between LS patients with ventricular hypertrophy and patients with familial HCM, it is clear that the pathophysiology and dynamics of HCM in LS differ from ventricular hypertrophy of other causes. To date, it is unclear whether the genotype may influence the clinical course in LS patients with HCM, especially because many of the affected individuals described in the literature are children and no clear risk figures based on a follow-up patient cohort study of a sufficient size is available. However, anecdotal reports provide enough evidence to state that long-term prognosis seems benign in LS patients with only mild cardiac abnormalities, whereas HCM in LS is indeed associated with a risk of fatal cardiac events as seen in primary HCM (Limongelli *et al*., 2008; Martínez-Quintana and Rodríguez-González, 2012a).

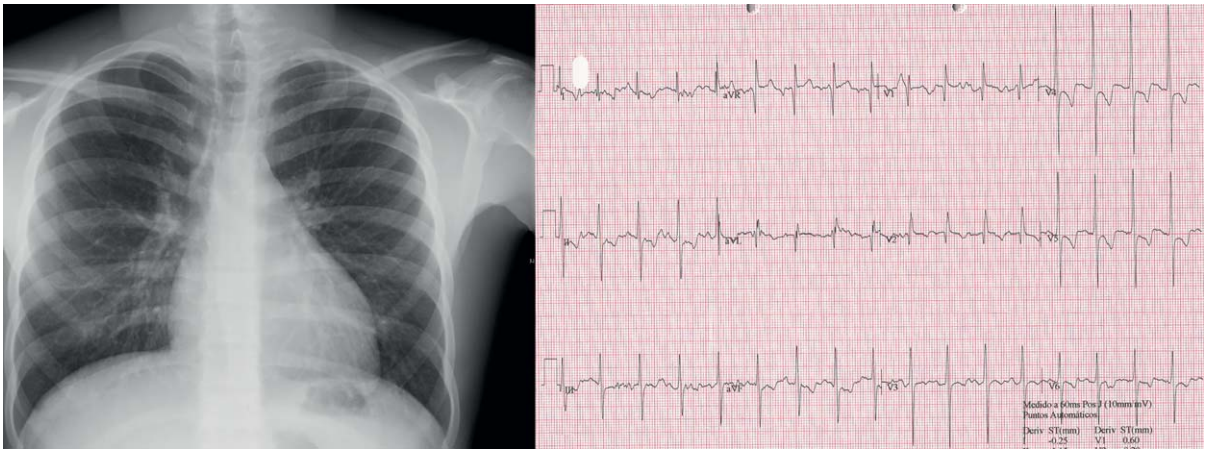
However, pulmonary stenosis (valvular, isolated infundibular, valvular and infundibular or with a dysplastic pulmonary valve) is less frequent than left ventricular hypertrophy in patients with LS (23% of the patients). Meanwhile, abnormalities of the aortic valve (23% of the cases) such as mild aortic regurgitation, discrete subaortic stenosis or aortic valve dysplasia and alterations of the mitral valve (38% of the patients) such as mitral valve prolapse are more frequent. Furthermore, coronary abnormalities, such as dilatation of the coronary arteries, abnormalities of the left ventricular shape, segmental wall motion, apical aneurysm of the left ventricle, noncompacted left ventricle, isolated left ventricular enlargement, atrioventricular septal defects and ventricular septal defects are also observed (Martínez-Quintana and Rodríguez-González, 2012b).

Hearing Loss

Sensorineural deafness occurs in approximately 15–25% of patients with LS (Sarkozy *et al*., 2004) and may be unilateral but can also be profound. Most patients have deafness diagnosed at birth or during childhood although some cases may develop in adulthood. Amplification of the sound with hearing aids or cochlear implants may help many of these patients with their auditory deficits (**Figure 3a**).

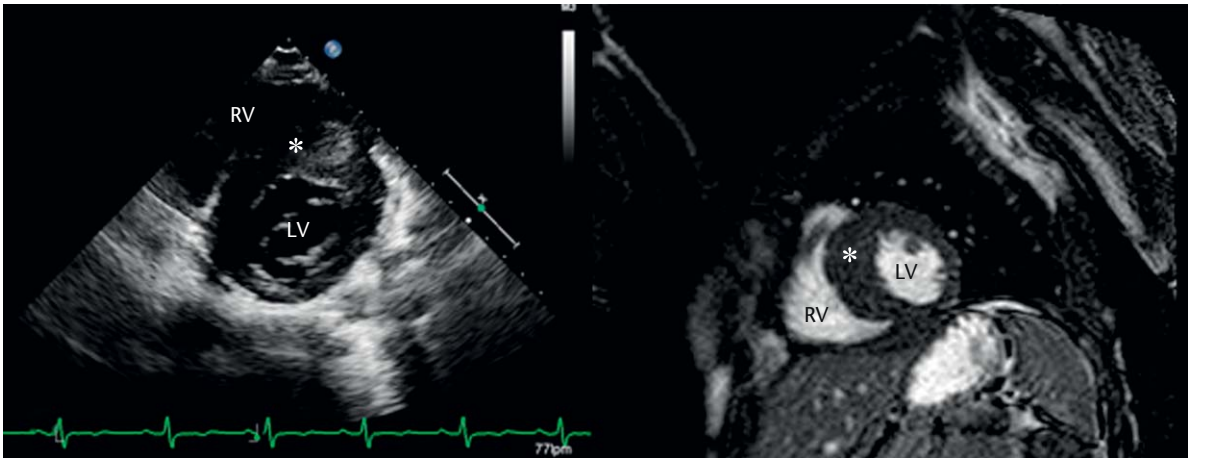
Growth and Neurological Anomalies

LS patients may have postnatal growth retardation and short stature (fewer than 50% of affected persons).



(a) (b)

Figure 4 (a) Posteroanterior chest X-ray showing mild vertical displacement of the left ventricular apex in the context of left ventricular hypertrophy in a patient with LEOPARD syndrome. (b) Twelve-lead ECG showing high R waves in the right precordial leads with ST depression in the context of LVH and systolic ventricular overload.



(a) (b)

Figure 5 Two-dimensional echocardiogram performed in the parasternal short-axis view (a) and coronal view of magnetic resonance angiography (b) showing left ventricular hypertrophy involving the intraventricular septum (asterisk). RV: right ventricle, LV: left ventricle.

Meanwhile, mild learning difficulties are reported in approximately 30% of the cases. However, mental retardation is rare and usually of mild degree (Sarkozy *et al*., 2008). Besides, nystagmus, hypotonia, hyposmia, autism, seizures and abnormalities in electroencephalograms may also be seen.

Skeletal and Genitourinary Tract Anomalies

Pectus carinatum or excavatum is frequently seen in LS patients. However, mandibular prognathism, winging of the scapulae, scoliosis, joint hyperflexibility, rib anomalies, syndactyly, cervical spine fusion and agenesis/supernumerary teeth are uncommon (Sarkozy *et al*., 2008). However, unilateral or more likely bilateral cryptorchidism occurs in approximately 50% of males, with hypospadia and genital hypoplasia also being frequent.

Carcionogenesis

Mutations in RAS genes can lead to the production of permanently activated RAS proteins. This can cause unintended and overactive signalling inside the cell, even in the absence of incoming signals. As these signals result in cell growth and division, overactive RAS signalling can ultimately lead to cancer. Although some haematologic tumours have been reported (Laux *et al*., 2008; Uçar *et al*., 2006; Schrader *et al*., 2009) in patients with LS the association is inconclusive (Kratz *et al*., 2011). Despite this, LS patients should be closely monitored for malignancy, particularly during their childhood.

Differential Diagnosis: From NS to LS

NS is a relatively common, clinically variable and genetically heterogeneous developmental disorder characterised by postnatally reduced growth, facial dysmorphia, cardiac abnormalities, variable cognitive deficits, cryptorchidism, bleeding tendency and, rarely, predisposition to haematologic malignancies during childhood (Tartaglia *et al*., 2010).

Many of the phenotypic abnormalities seen in LS, such as short stature, facial dysmorphism and skeletal anomalies, resemble NS phenotypically. However, LS is characterised by multiple lentigines dispersed throughout the body and café-au-lait spots. Moreover, their cardiac phenotypes are quite distinct. In fact, though HCM is the most frequent heart defect found in LS, the most common cardiac manifestation in NS is pulmonic stenosis resulting from dysplastic valve leaflets, followed (less frequently) by HCM, mitral stenosis and atrial, ventricular and atrioventricular septal defects, or (rarely) by double outlet right ventricle (Martínez-Quintana and Rodríguez-González, 2013). Despite these differences, and because of the common features between the two syndromes, some researchers denominate the LS as Noonan-like syndrome with multiple lentigines.

In relation to genetic mutations, NS is caused by mutations in the PTPN11, SOS1, KRAS, RAF1, BRAF and MEK1 (MAP2K1) genes, accounting for approximately 70% of affected individuals. Of these mutations seen in NS, the majority are due to alterations in the PTPN11 gene (50% of the patients). Meanwhile, PTPN11 mutations are observed in up to 90% of the patients with LS occurring mutations in the RAF1 and BRAF genes in 5% of cases (Lauriol and Kontaridis, 2011; Tartaglia *et al*., 2010). Nonetheless, the point mutations identified in PTPN11 that are associated with NS are distinct from those

associated with LS and therefore with different biochemical properties: gain-of-function mutations in PTPN11, characterised by an increase in physiological activity of the mutated protein, are more frequent in NS patients (Musante *et al*., 2003; Niihori *et al*., 2006) whereas a loss-of-function or dominant-negative mutations in PTPN11 are more prevalent in patients with LS (Kontaridis *et al*., 2006; Tartaglia *et al*., 2006). These gain-of-function and loss-of-function mutations may explain the differences in phenotypes between these two syndromes. It is unclear, however, how the inactivating and activating mutations in the same gene observed in LS and NS, respectively, can lead to similar phenotypic manifestations. Anyway, it is believed that LS mutations weaken the intramolecular interaction between the *N*-SH2 and phosphatase domains, leading to a change in SHP2 molecular switching mechanism. Consequently, SHP2 mutants bind upstream activators preferentially, are hypersensitive to growth factor stimulation and stay longer with scaffolding adaptors, thus prolonging substrate turnover, which compensates for the reduced phosphatase activity (Yu *et al*., 2013).

Conclusions

LS is an acronym for the cardinal features lentigines, ECG conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth and sensorineural deafness, a disorder which is part of a newly classified family of autosomal dominant syndromes termed 'RASopathies', which are caused by germline mutations in components of the RAS-MAPK signal transduction pathway.

Clinically, lentigines are the most prominent manifestation of LS and are found in more than 90% of the patients. However, other disorders, such as HCM, occur frequently and represent a potentially life-threatening problem in these patients.

PTPN11 mutations, located on chromosome 12q24, are observed in up to 90% of patients with LS. Meanwhile, mutations in the RAF1 and BRAF gene may be seen in up to 5% of cases. However, to date, it is unclear whether the genotype may influence the clinical course in patients with LS.

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