Clinical and molecular characterization of children with Noonan syndrome and other RASopathies in Argentina

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ABSTRACT

Introduction. RASopathies are a set of syndromes with phenotypic overlapping features caused by gene mutations involved in the RAS/MAPK pathway. They are autosomal dominantly inherited and share common clinical characteristics, including short stature, craniofacial dysmorphisms, congenital heart disease, ectodermal manifestations, and a higher risk for cancer. A molecular diagnosis is a key factor.

Objective. To identify *PTPN11*, *SOS1*, *RAF1*, *BRAF*, and *HRAS* mutations and compare the main clinical characteristics of patients with molecular confirmation.

Population and methods. Children with a clinical diagnosis of RASopathy assessed between August 2013 and February 2017.

Results. Mutations were identified in 71 % (87/122) of patients. The molecular test confirmed diagnosis in 73 % of patients with Noonan syndrome. The most prevalent mutation was c.922A>G (p.Asn308Asp) in the *PTPN11* gene. A previously undescribed variant in *RAF1* was detected: c.1467G>C (p.Leu489Phe). Cardiofaciocutaneous syndrome was confirmed in 67 % of cases with *BRAF* mutations. Costello syndrome and Noonan syndrome with multiple lentigines were confirmed in all cases.

Conclusion. The confirmation of clinical diagnosis allowed for a more accurate differential diagnosis. The prevalence of *PTPN11* (58 %), *SOS1* (10 %), and *RAF1* mutations (5 %) in children with Noonan syndrome, of *PTPN11* mutations (100 %) in those with Noonan syndrome with multiple lentigines, of *BRAF* mutations (67 %) in those with cardiofaciocutaneous syndrome, and of *HRAS* mutations (100 %) in those with Costello syndrome was determined.

Key words: *RASopathies, Noonan syndrome,* PTPN11, RAF1, *Argentina.*

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GLOSSARY

CFC syndrome: cardiofaciocutaneous syndrome.
CS: Costello syndrome.
HCM: hypertrophic cardiomyopathy.
NS: Noonan syndrome.
NSML: Noonan syndrome with multiple lentigines.
PS: pulmonary stenosis.

INTRODUCTION

RASopathies are a set of syndromes with phenotypic overlapping features, including Noonan syndrome (NS), Noonan syndrome with multiple lentigines (NSML), Costello syndrome (CS), cardiofaciocutaneous (CFC) syndrome, and neurofibromatosis-Noonan syndrome. RASopathies are caused mainly by gain-of-function mutations in the genes involved in the RAS/MAPK signaling pathway. This is implied in several biological functions, such as the regulation of cell proliferation and cell survival and differentiation.[1]

These syndromes are autosomal dominantly inherited and share some clinical characteristics.

NS (OMIM 163950) is the most common one in this set, with an incidence of 1:1000 to 1:2500 live births.[1] Its main characteristics include short stature, congenital heart disease, craniofacial dysmorphisms, and thoracic malformations.[2] Sometimes, intellectual disability, hearing loss, short neck, clotting alterations, and cryptorchidism (in males) may occur.[3] Children with NS have a higher risk for developing different types of cancer.[4] NS is caused by the following gene mutations: *PTPN11* (50 %), *SOS1* (10-15 %), *RAF1* (5-15 %), *RIT1* (4-

9 %), *KRAS* (<5 %), *NRAS* (<1 %), *BRAF* (<1 %), *SHOC2* (<1 %), *MEK1* (<1 %), and *CBL* (<1 %).[5-9] Approximately 70-80 % of patients with NS have these gene mutations.[10] The causative mutations have not been identified in the other 20-30 %.[11] *De novo* mutations account for 60 % of NS cases.[12]

The clinical characteristics of NSML (previously known as LEOPARD syndrome, OMIM 151100) include the presence of multiple lentigines, ECG abnormalities, ocular hypertelorism, pulmonary stenosis (PS), genital abnormalities, delayed growth, and hearing loss. It is caused by *PTPN11* (90 %), *RAF1* (5 %), and *BRAF* (5 %) mutations.[6]

CFC syndrome (OMIM 115150) is characterized by craniofacial dysmorphisms, neurological development delay, congenital heart disease, and ectodermal and musculoskeletal manifestations. It is caused by *de novo* mutations in the *BRAF* gene (75 %) and the *MEK1*/*MEK2* gene (25 %). *KRAS* mutations were reported in a lower percentage of cases (<1 %).[13,14]

CS (OMIM 218040) is one of the less common RASopathies. Its characteristics include intellectual disability, high birth weight, difficulty sucking in the neonatal period, perioral and perianal papillomas, and deep palmar and plantar creases. Patients with CS have a 13 % probability of developing tumors.[15] It is caused by heterozygous mutations in the *HRAS* proto-oncogene in more than 80 % of cases.[16]

Given the overlapping clinical features of these syndromes and their variable expression, molecular testing is critical to establish an accurate diagnosis. The objective of this study was to identify *PTPN11*, *SOS1*, *RAF1*, *BRAF*, and *HRAS* mutations in children with RASopathies and compare the main clinical characteristics of patients with molecular confirmation.

POPULATION AND METHODS

This was a cross-sectional, observational, and descriptive study that included patients with a clinical diagnosis of RASopathy[2,9,17] assessed between August 2013 and February 2017 by the Department of Genetics of Hospital de Pediatría Garrahan. Two clinical geneticists recorded the presence of dysmorphisms, congenital heart disease, skeletal and kidney abnormalities, ectodermal manifestations, cryptorchidism, hearing loss, clotting alterations, splenomegaly, lymphatic dysplasia, intellectual disability, and cancer.

Anthropometric parameters were analyzed by sex and age, and are described as standard deviation (SD). The Argentine weight and height charts were used as the reference population,[18] and the Nellhaus chart, for head circumference.[19]

A written informed consent was approved by the hospital's Ethics Committee and obtained from patients and/or their parents.

Molecular analysis

Deoxyribonucleic acid (DNA) samples from peripheral blood lymphocytes were analyzed for mutations using the Sanger sequencing technique (ABI BigDye Terminator Sequencing Kit V1.1; Applied Biosystems) with an automated capillary sequencer (ABI 3130, Applied Biosystems). Exons 2, 3, 4, 7, 8, 12, and 13 of the *PTPN11* gene, exons 6 and 10 of the *SOS1* gene, exons 7, 14, and 17 of the *RAF1* gene, exons 6, 12, and 15 of the *BRAF* gene, and exon 2 of the *HRAS* gene were analyzed. Sequencing was done by stages, in order of relevance, according to the mutation frequency described in the main exons of each gene.[6,9,13,14,20] The *HRAS* gene was tested in patients with suspected CS.[16]

Statistical analysis

Fisher's exact test was done. A p value < 0.05 was considered statistically significant.

RESULTS

A total of 122 patients (56 females and 66 males) diagnosed with RASopathy were assessed. The median age at the time of clinical diagnosis was 6 years (range: 0-19 years). Most patients were of European descent, chiefly Spanish and Italian. Mutations were detected in 87 patients (71 %). Initially, 100 patients received a clinical diagnosis of NS; 16, CFC syndrome; 3, NSML; and 3, CS.

The mutation identified in 3 patients led to a modification in the initial diagnosis. One of them was assessed at 2 years old due to dysmorphisms, short stature, and ectodermal manifestations and received an initial diagnosis of CFC syndrome. This patient was reassessed at 16 years old and showed no intellectual disability or heart disease. A new variant in the *RAF1* gene was detected and the diagnosis was changed to NS. In another 2-year-old patient without skin manifestations at the time of assessment and with an initial diagnosis of NS, the p.Thr468Met mutation in the *PTPN11* gene was detected, which had been previously associated with NSML; the subsequent assessment of his father showed

multiple lentigines at the time of the physical examination and this changed the diagnosis to NSML. Finally, there was a patient with a clinical diagnosis of CS, but the detection of a *BRAF* mutation led to changing it to CFC syndrome.

In addition, a patient with clinical diagnosis of CFC syndrome due to ectodermal manifestations, such as keratosis pilaris, curly hair, and sparse eyebrows, without cognitive deficit or congenital heart disease, had the c.806C>T mutation (p.Met269Thr) in the *SOS1* gene, which is rarely associated with CFC syndrome.

In five patients with a negative molecular test, follow-up allowed to establish a diagnosis other than RASopathy. The clinical reassessment determined a total of 96 patients with a diagnosis of NS; 15, CFC syndrome; 4, NSML; and 2, CS.

The molecular test confirmed the diagnosis in 71/96 patients with NS (73 %); 56 (58 %) had *PTPN11* mutations; 10, (10 %) *SOS1* mutations; and 5 (5 %), *RAF1* mutations. No mutations were detected with the methodology used here in the other 25 patients. All patients with NSML had *PTPN11* mutations. CFC syndrome was confirmed in 10/15 cases with *BRAF* mutations. CS was confirmed in 2 patients with *HRAS* mutations.

Among patients in whom a mutation was detected, 72 corresponded to sporadic cases and 15, to familial cases; transmission was maternal in 11 of them.

TABLE 1. *Clinical characteristics of patients with RASopathies*

	Noonan syndrome (N = 96)				**Noonan syndrome with multiple lentigines (N = 4)**	**Cardiofaciocutaneous syndrome (N = 15)**		**Costello syndrome (N = 2)**
	PTPN11	***SOS1***	***RAF1***	**No mutations detected**	***PTPN11***	***BRAF***	**No mutations detected**	***HRAS***
	(n = 56)	**(n = 10)**	**(n = 5)**	**(n = 25)**	**(n = 4)**	**(n = 10)**	**(n = 5)**	**(n = 2)**
Male/female	(28 : 28)	(8 : 2)	(2 : 3)	(15 : 10)	(3 : 1)	(5 : 5)	(2 : 3)	(0 : 2)
Craniofacial dysmorphisms	56 (100 %)	10 (100 %)	5 (100 %)	25 (100 %)	4 (100 %)	10 (100 %)	5 (100 %)	2 (100 %)
Heart disease	44 (78 %)	9 (90 %)	4 (80 %)	16 (64 %)	4 (100 %)	7 (70 %)	4 (80 %)	2 (100 %)
Pulmonary valve stenosis (PVS)	30/44 (68 %)	6/9 (67 %)	1/4 (25 %)	10/16 (63 %)	0	3/7 (42 %)	1/4 (25 %)	1 (50 %)
Hypertrophic cardiomyopathy (HCM)	1/44 (2 %)	-	2/4 (50 %)	2/16 (13 %)	4 (100 %)	-	2/4 (50 %)	
PS and HCM	6/44 (14 %)	1/9 (11 %)	-	2/16 (13 %)	-	2/7 (29 %)	-	-
Other structural defects*	7/44 (16 %)	2/9 (22 %)	1/4 (25 %)	2/16 (13 %)	-	2/7 (29 %)	1/4 (25 %)	1 (50 %)
Thoracic malformations	43 (77 %)	9 (90 %)	3 (60 %)	19 (76 %)	3 (75 %)	9 (90 %)	4 (80 %)	2 (100 %)
Scoliosis	3 (5 %)	-	1 (20 %)	1 (4 %)	-	1 (10 %)	-	-
Cubitus valgus	20 (36 %)	4 (40 %)	1 (20 %)	9 (36 %)	2 (50 %)	2 (20 %)	3 (60 %)	-
Ectodermal manifestations (skin/hair)	18 (32 %)	5 (50 %)	5 (100 %)	13 (52 %)	3 (75 %)	10 (100 %)	5 (100 %)	2 (100 %)
Cryptorchidism (males)	18/28 (64 %)	4/8 (50 %)	-	5 (20 %)	-	1 (10 %)	2 (40 %)	n/a
Sensorineural hearing loss	7 (13 %)	-	1 (20 %)	-	1 (25 %)	4 (40 %)	1 (20 %)	-
Clotting alterations	8 (14 %)	1 (10 %)	-	4 (16 %)	-	-	-	-
Splenomegaly	6 (11 %)	-	1 (20 %)	1 (4 %)	-	-	-	-
Lymphatic dysplasia	4 (7 %)	1 (10 %)	-	5 (20 %)	-	1 (10 %)	1 (20 %)	1 (100 %)
Cancer	3 (5 %)	-	-	-	-	-	-	-
Height (-2 SD)	34 (61 %)	4 (40 %)	2 (40 %)	9 (36 %)	-	7 (70 %)	3 (60 %)	2 (100 %)
Microcephalus	16 (29 %)	2 (20 %)	1 (20 %)	-	-	-	-	1 (50 %)
Macrocephaly	-	-	1 (20 %)	1 (4 %)	-	3 (30 %)	3 (60 %)	-
Overall developmental delay or intellectual disability	33 (59 %)	4 (40 %)	2 (40 %)	16 (64 %)	-	10 (100 %)	3 (60 %)	2 (100 %)
Kidney disorders	5 (9 %)	1 (10 %)	-	4 (16 %)	1 (25 %)	3 (30 %)	1 (20 %)	

* Atrial and ventricular septal defect, bicuspid aortic valve, aortic stenosis, coarctation of the aorta, tetralogy of Fallot.

The clinical characteristics are summarized in *Table 1*. The 96 patients with NS had facial dysmorphisms; 51 % of them had short stature, which was more common among those with *PTPN11* mutations. Also, 76 % of patients had heart disease, with PS as the most frequent condition. Hypertrophic cardiomyopathy (HCM), aortic stenosis, tetralogy of Fallot, atrial septal defect, and ventricular septal defect were also detected.

The comparison of the main phenotypic features of patients with molecular confirmation of NS and NSML (*Table 2*) showed that the latter had a strong association with HCM, a smaller presence of short stature and overall developmental delay or intellectual disability ($p < 0.05$). Skin manifestations were observed in 75 % of patients with NSML, but no significant differences were observed in terms of this clinical characteristic when compared to NS patients (*Table 2*).

A strong relation between patients with CFC syndrome and ectodermal manifestations and hearing loss was observed in comparison with those who had NS ($p < 0.05$) (*Table 2*).

No significant differences were seen in relation to ectodermal manifestations, short stature, and overall developmental delay or intellectual disability when comparing clinical manifestations between NS patients with *PTPN11* and *SOS1* mutations. NS patients with *RAF1* mutations had a higher incidence of HCM compared to those with *PTPN11* mutations ($p = 0.015$).

Molecular characteristics and distribution of mutations

The greatest number of mutations was detected in the *PTPN11* gene (60), followed by the *SOS1* (10) and *BRAF* (10) genes (*Table 3*). *RAF1* and *HRAS* mutations were detected in 5 and 2 patients, respectively. All the mutations that were detected had been previously reported in the bibliography, except for a new variant in the *RAF1* gene, c.1467G>C (p.Leu489Phe). It was registered in the Leiden Open Variation Database (LOVD).[21]

Familial cases were observed in 12 patients with a mutation in the *PTPN11* gene, 2 in the *SOS1* gene, and 1 in the *RAF1* gene (*Table 3*). Also, 75 % of mutations detected in the *PTPN11* gene were located in exons 3, 8, and 13.

In silico analyses were done to predict the functional effect of the new variant detected in the *RAF1* gene, c.1467G>C. The variant was analyzed using different biocomputing tools that assessed the functional effects of single-nucleotide variants in human beings (Mutation Taster, PolyPhen2, and SIFT). The variant was identified as probably deleterious (score: 0.997) with Polyphen; pathogenic, with Mutation

TABLE 2. *Comparison of the main clinical characteristics of patients with Noonan syndrome, cardiofaciocutaneous syndrome, and Noonan syndrome with multiple lentigines*

	NS (N = 71)	CFCS (N = 10)	*p*	NSML (N = 4)	*p*
Craniofacial dysmorphisms	71 (100 %)	10 (100 %)		4 (100 %)	
Heart disease	57 (80 %)	7 (70 %)	0.68	4 (100 %)	0.58
Pulmonary valve stenosis (PVS)	37/57 (65 %)	3/10 (42 %)	0.41	0	**0.02**
Hypertrophic cardiomyopathy (HCM)	3/57 (5 %)	0/10	1	4 (100 %)	**0.00006**
PS and HCM	7/57 (12 %)	2/10 (29 %)	0.25	0	1
Other*	10/57 (18 %)	2/10 (29 %)	0.61	0	0.6
Thoracic malformations	55 (77 %)	9 (90 %)	0.45	3 (75 %)	1
Cubitus valgus	25 (35 %)	2 (20 %)	0.48	2 (50 %)	0.62
Ectodermal manifestations (skin/hair)	28 (39 %)	10 (100 %)	**0.0003**	3 (75 %)	0.3
Cryptorchidism (males)	22/38 (58 %)	1/5 (20 %)	0.17	0/3	0.09
Sensorineural hearing loss	8 (11 %)	4 (40 %)	**0.04**	1 (25 %)	0.41
Clotting alterations	9 (13 %)	0	0.36	0	1
Splenomegaly	7 (9 %)	0	0.59	0	1
Lymphatic dysplasia	5 (7 %)	0	1	0	1
Cancer	3 (4 %)	0	1	0	1
Height (-2 SD)	40 (56 %)	7 (70 %)	0.51	0	**0.04**
Overall developmental delay or intellectual disability	55 (77 %)	10 (100 %)	0.2	0	**0.04**
Kidney disorders	10 (14 %)	3 (30 %)	0.35	1 (25 %)	0

* Atrial and ventricular septal defect, bicuspid aortic valve, aortic stenosis, coarctation of the aorta, tetralogy of Fallot.
NS: Noonan syndrome; CFCS: cardiofaciocutaneous syndrome; NSML: Noonan syndrome with multiple lentigines.

TABLE 3. *Mutations detected in patients with RASopathy*

Gen	Exon	Nucleotide change	Amino acid change	Domain	N	Initial diagnosis	Occurrence	Final diagnosis
PTPN11	2	c.124A/G	p.*Thr*42*Ala*	Regulatory	1	NS	Sporadic	NS
(N = 60)	3	c.179 G/C	p.*Gly*60*Ala*	Regulatory	1	NS	Familial	NS
		c.181 G/A	p.*Asp*61*Asn*	Regulatory	3	NS	Sporadic	NS
		c.182A/C	p.*Asp*61*Ala*	Regulatory	1	NS	Sporadic	NS
		c.184T/G	p.*Tyr*62*Asp*	Regulatory	2	NS	Sporadic	NS
		c.188A/G	p.*Tyr*63*Cys*	Regulatory	1	NS	Sporadic	NS
		c.205G/C	p.*Glu*69*Gln*	Regulatory	1	NS	Sporadic	NS
		c.214G/T	p.*Ala*72*Ser*	Regulatory	1	NS	Sporadic	NS
		c.215C/G	p.*Ala*72*Gly*	Regulatory	1	NS	Sporadic	NS
		c.218C/T	p.*Thr*73*lle*	Regulatory	1	NS	Sporadic	NS
		c.228G/C	p.*Glu*76*Asp*	Regulatory	2	NS	Sporadic	NS
		c.236A/G	p.*Gln*79*Arg*	Regulatory	1	NS	Sporadic	NS
					2		Familial	NS
	4	c.417G/C	p.*Glu*139*Asp*	Bridge region	3	NS	Sporadic	NS
					2		Familial	NS
	7	c.836A/G	p.*Tyr*279*Cys*	Active center	2	NSML	Sporadic	NSML
		c.844A/G	p.*Ile*282*Val*	Active center	2	NS	Sporadic	NS
		c.846C/G	p.*Ile*282*Met*	Active center	1	NS	Sporadic	NS
		c.853T/C	p.*Phe*285*Leu*	Active center	2	NS	Sporadic	NS
	8	c.922A/G	p.*Asn*308*Asp*	Active center	8	NS	Sporadic	NS
					4		Familial	NS
		c.923A/G	p.*Asn*308*Ser*	Active center	1	NS	Sporadic	NS
	12	c.1403C/T	p.*Thr*468*Met*	Active center	1	NS	Familial	NSML
	13	c.1471C/T	p.*Pro*491*Ser*	Active center	1	NS	Familial	NS
		c.1472C/A	p.*Pro*491*His*	Active center	1	NS	Sporadic	NS
		c.1472C/T	p.*Pro*491*Lys*	Active center	1	NS	Sporadic	NS
		c.1507G/C	p.*Gly*503*Arg*	Active center	3	NS	Sporadic	NS
		c.1510A/G	p.*Met*504*Val*	Active center	7	NS	Sporadic	NS
					1		Familial	NS
		c.1529A/C	p.*Gin*510*Pro*	Active center	1	NSML	Sporadic	NSML
		c.1530G/C	p.*Gin*510*His*	Active center	1	NS	Sporadic	NS
SOS	6	c.797C/A	p.*Thr*266*Lys*	DH	2	NS	Sporadic	NS
(N = 10)		c.806C/T	p.*Met*269*Thr*	DH	1	CFCS	Sporadic	CFCS/NS
	10	c.1300G/A	p.*Gly*434*Arg*	PH	1	NS	Familial	NS
		c.1322G/A	p.*Cys*441*Tyr*	PH	1	NS	Sporadic	NS
		c.1642A/C	p.*Ser*548*Arg*	PH-REM linker	1	NS	Sporadic	NS
		c.1649T/C	p.*Leu*550*Pro*	PH-REM linker	1	NS	Sporadic	NS
		c.1654A/G	p.*Arg*552*Gly*	PH-REM linker	2	NS	Sporadic	NS
		c.1656G/C	p.*Arg*552*Ser*	PH-REM linker	1	NS	Familial	NS
RAF1	7	c.770C/T	p.*Ser*257*Leu*	CR2	1	NS	Sporadic	NS
(N = 5)		c.788T/G	p.*Val*263*Gly*	CR2	1	NS	Sporadic	NS
	14	c.1423T/C	p.*Phe*475*Leu*	Activation	1	NS	Sporadic	NS
		c.1467G/C	p.*Leu*489*Phe**	Activation	1	CFCS	Familial	NS
	17	c.1837C/G	p.*Leu*613*Val*	Activation	1	NS	Sporadic	NS
BRAF	6	c.735A/C	p.*Leu*245*Phe*	CR1	1	CFCS	Sporadic	CFCS
(N = 10)		c.736G/C	p.*Ala*246*Pro*	CR1	2	CFCS	Sporadic	CFCS
		c.770A/G	p.*Gln*257*Arg*	CR1	5	CFCS/CS	Sporadic	CFCS
	12	c.1495A/G	p.*Lys*499*Glu*	Activation	1	CFCS	Sporadic	CFCS
	15	c.1787G/T	p.*Gly*596*Val*	Activation	1	CFCS	Sporadic	CFCS
HRAS	2	c.34G>A	p.*Gly*12*Ser*	Active center	1	CS	Sporadic	CS
(N = 2)		c.35G>C	p.*Gly*12*Ala*	Active center	1	CS	Sporadic	CS

* New mutation.

NS: Noonan syndrome; CFCS: cardiofaciocutaneous syndrome; NSML: Noonan syndrome with multiple lentigines; CS: Costello syndrome; DH: Dbl homology domain; PH: pleckstrin homology domain; REM: RAS exchanger motif domain; CR: conserved region.

Taster; and deleterious (score: -3.605), with SIFT. This variant had been previously detected in the skin of a patient with malignant melanoma (COSM5398071) and has been described in the COSMIC database (Catalogue of Somatic Mutations in Cancer).

DISCUSSION

In this study, mutations were detected in 71 % of patients with NS and other RASopathies. The most prevalent mutation in our cohort was p.Asn308Asp, in the *PTPN11* gene. This is also one of the most prevalent mutations in the European population,[20,22] but it has not been reported in the Chilean or Brazilian population.[23,24] This may be due to the descent of Argentine patients. The ethnic origin of our population is the result of mixed native genes and genes from, mostly, Europe's Mediterranean countries, especially Spain and Italy and, to a lesser extent, Central and Eastern Europe and Middle East.[25]

In relation to NS, the molecular test confirmed the clinical diagnosis in 73 % of cases, which is consistent with the bibliography.[26] In this cohort, only 15 % of patients corresponded to familial cases, lower than what has been published by Tartaglia et al., who reported that 30-75 % of NS cases were familial.[27] This may be because not all parents were available for the clinical assessment and the genetic analysis.

Patients with NS and *PTPN11* or *SOS1* mutations typically have a high incidence of PS and a lower prevalence of HCM.[5] In our study, similar results were reported: 68 % of patients with *PTPN11* mutations had PS; and 2 %, HCM. Among patients with *SOS1* mutations, 90 % had a heart disease, with PS being the most prevalent one.

SOS1 mutations are associated with a phenotype within the clinical spectrum of NS, but it is characterized by a high prevalence of ectodermal manifestations, a low incidence of cognitive deficit, and short stature.[14] In our cohort, ectodermal manifestations were present in 50 % of NS patients with *SOS1* mutations; most of them had sparse eyebrows. Short stature and intellectual disability were observed in 40 % of patients. This may not have been similar to what has been reported in the bibliography[14] due to the relatively small number of patients with *SOS1* mutations and the young age of assessed patients.

RAF1 mutations have been associated with HCM, arrhythmia, and intellectual disability.[6] Some previous publications have proposed that approximately 85 % of patients with *RAF1* mutations tend to develop HCM.[28] For this reason, it is very important that these patients receive an intensive cardiac follow-up. A previously undescribed variant in this gene, c.1467G>C (p.Leu489Phe), was detected, in which both the *in silico* analysis and the patient's and mother's clinical characteristics help to link it to the syndrome.

As described in the bibliography, patients with RASopathies typically have a greater risk for tumor development. This characteristic was also observed in our cohort, and 3 NS patients who had *PTPN11* mutations developed acute lymphoblastic leukemia and paravertebral ganglioneuroma.

In NSML, the main clinical characteristics include skin manifestations, which sometimes do not appear until after puberty. In our cohort, only 1 patient did not have skin manifestations but he was only 2 years old at the time of assessment.

Among patients with a clinical diagnosis of CFC syndrome, the molecular test confirmed diagnosis in 67 % of cases, which is consistent with previous publications.[13]These patients had a high rate of intellectual disability and ectodermal manifestations. These two characteristics were also prevalent among the patients in our cohort. This study found a high incidence of hearing loss (40 %), which, although it was not a distinguishing feature of CFC syndrome, had already been reported by Carcavillaet al.[29]

As mentioned in the results, in one of our patients diagnosed with CFC syndrome, a mutation was identified in the *SOS1* gene: c.806C>T (p.Met269Thr). Although this gene was not associated with CFC syndrome, Tartaglia et al.[30] reported on patients with the CFC syndrome phenotype who had *SOS1* mutations and proposed that the clinical characteristics of NS and CFC syndrome overlapped. Our case supported the observations of those authors.

The Sanger sequencing technique, which was used in this study and is available in our hospital, did not detect mutations in 29 % of cases. Although this is a sensitive and specific methodology, it is a tedious, slow, and costly method to study genetically heterogeneous syndromes. Therefore, a next generation sequencing (NGS) panel focused on a set of RASopathy-associated genes is a more adequate and cost-effective option to diagnose these syndromes.

To sum up, this study determined, in Argentine children, the prevalence of mutations

in the following genes: *PTPN11* (58 %), *SOS1* (10 %), and *RAF1* (5 %) in NS; *PTPN11* (100 %) in NSML; *BRAF* (67 %) in CFC syndrome; and *HRAS* (100 %) in CS. Given the overlapping clinical characteristics of these syndromes, especially during childhood, molecular testing is a critical tool for diagnostic confirmation. In addition, it helps to provide more accurate guidance on prognosis, the adequate management of specific complications, e.g., cardiac, hematological or oncological, the risk for recurrence, and the analysis of other family members at risk. ■

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