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RASopathies: Clinical Diagnosis in the First Year of Life

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Cardiofaciocutaneous syndrome · Costello syndrome · Genotype-phenotype correlations · LEOPARD syndrome · Noonan syndrome · Noonan-like syndrome with loose anagen hair

Abstract
Diagnosis within Noonan syndrome and related disorders (RASopathies) still presents a challenge during the first months of life, since most clinical features used to differentiate these conditions become manifest later in childhood. Here, we retrospectively reviewed the clinical records referred to the first year of life of 57 subjects with molecularly confirmed diagnosis of RASopathy, to define the early clinical features characterizing these disorders and improve our knowledge on natural history. Mildly or markedly expressed facial features were invariably present. Congenital heart defects were the clinical issue leading to medical attention in patients with Noonan syndrome and LEOPARD syndrome. Feeding difficulties and developmental motor delay represented the most recurrent features occurring in subjects with cardiofaciocutaneous syndrome and Costello syndrome. Thin hair was prevalent among *SHOC2* and *BRAF* mutation-positive infants. Café-au-lait spots were found in patients with LS and *PTPN11* mutations, while keratosis pilaris was more common in individuals with *SOS1*, *SHOC2* and *BRAF* mutations. In conclusion, some characteristics can be used as hints for suspecting a RASopathy during the first months of life, and individual RASopathies may be suspected by analysis of specific clinical signs. In the first year of life, these include congenital heart defects, severity of feeding difficulties and delay of developmental milestones, hair and skin anomalies, which may help to distinguish different entities, for their subsequent molecular confirmation and appropriate clinical management.

In the last 10 years, germline mutations in a number of genes coding transducers and modulatory proteins participating in the RAS-MAP kinase-signaling pathway have been causally linked to Noonan syndrome (NS) and a group of clinically related disorders, the so-called neuro-cardio-facio-cutaneous syndromes or RASopathies [Schubbert et al., 2007; Tidyman and Rauen, 2009; Tartaglia et al., 2010]. These disorders are characterized by facial dysmorphism, a wide spectrum of cardiac dis-

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ease, postnatal reduced growth, ectodermal and skeletal defects, and variable cognitive deficits. Mutations in *PTPN11, SOS1, KRAS, NRAS, RAF1* and *BRAF* genes have been documented to account for approximately 75% of individuals with NS [Tartaglia et al., 2001, 2007; Carta et al., 2006; Schubbert et al., 2006; Pandit et al., 2007; Razzaque et al., 2007; Roberts et al., 2007; Sarkozy et al., 2009a; Cirstea et al., 2010], the commonest and clinically most variable among these disorders [Allanson, 1987; Noonan, 1994; van der Burgt, 2007]. LEOPARD syndrome (LS) [Gorlin et al., 1969; Voron et al., 1976; Sarkozy et al., 2009b] and Noonan-like syndrome with loose anagen hair (NS/LAH) [Mazzanti et al., 2003], which phenotypically resemble NS, have been shown to be caused by mutations in the *PTPN11, RAF1* and *BRAF* [Digilio et al., 2002; Legius et al., 2002; Pandit et al., 2007; Sarkozy et al., 2009a], and *SHOC2* genes [Cordeddu et al., 2009], respectively, while defects in the *CBL* gene have been linked to a variable condition partially overlapping NS [Martinelli et al., 2010; Niemeyer et al., 2010; Perez et al., 2010]. Other disorders more severely affecting development and growth, but with clinical overlap with NS, include cardiofaciocutaneous syndrome (CFCS) [Reynolds et al., 1986; Roberts et al., 2006] and Costello syndrome (CS) [Costello, 1977; Hennekam, 2003; Gripp, 2005]. Both conditions are caused by defects in genes functionally related to those implicated in NS, the former being associated with mutations affecting the *KRAS, BRAF, MEK1* and *MEK2* genes [Niihori et al., 2006; Rodriguez-Viciana et al., 2006], and the latter being caused by a relatively narrow spectrum of *HRAS* mutations [Aoki et al., 2005].

In general, the clinical diagnosis of a condition with clinical traits fitting a specific RASopathy can be difficult in the first months of life, since most cardinal features utilized to categorize NS, LS, NS/LAH, CFCS and CS manifest later during childhood [Digilio et al., 2006a, 2007]. Nevertheless, a detailed definition of the neonatal phenotype and degree of variation characterizing these disorders would represent a clinically relevant tool to guide pediatricians and medical geneticists towards an earlier clinical diagnosis of general RASopathies and specific subtypes, prompting molecular characterization and more effective patient management and counseling.

Here, we review the clinical records referred to the first year of life of 57 patients clinically diagnosed as having a RASopathy and subsequently molecularly genotyped, in order to outline clinical features facilitating early diagnosis of these disorders, and add information on clinical history referred to the first months of life.

Patients and Methods

Patients, Molecular Analyses and Clinical Data Collection

From 1990 to 2010, 134 patients with clinical features fitting the RASopathy spectrum were evaluated in their first year of life. Within this cohort, genomic DNA obtained from circulating leukocytes was available for molecular analysis in 96 subjects, for whom no obvious selection bias was apparent. The entire coding sequence of the *PTPN11* gene was screened for mutations in all patients by single-strand conformation polymorphism analysis or denaturing high-performance liquid chromatography, as previously reported [Digilio et al., 2002; Tartaglia et al., 2002]. Fragments with an aberrant migration/elution pattern were sequenced. *PTPN11* mutation-negative samples were successively screened for mutations in the coding region of the *SOS1, RAF1, KRAS, NRAS, SHOC2, CBL, BRAF, MEK1, MEK2,* and *HRAS* genes by denaturing high-performance liquid chromatography analysis and bi-directional direct sequencing. Disease causative changes were identified in 57 patients (table 1). *PTPN11* mutations were detected in 31 subjects (54% of mutation-positive cases), allowing to obtain a molecularly confirmed diagnosis of NS and LS in 20 and 11 individuals, respectively. Heterozygous mutations in *SOS1* (n = 8, 14%), *RAF1* (n = 4, 7%), *BRAF* (n = 1, 2%), *NRAS* (n = 1, 2%) and *CBL* (n = 1, 2%) were found in subjects with a phenotype fitting in with NS. A missense *BRAF* mutation was documented in 4 patients with a diagnosis of CFCS (7%), and one *MEK2* mutation was found to occur in 1 additional CFCS case. Finally, mutations in *HRAS* and *SHOC2* were observed in 4 patients with CS (7%), and 2 subjects with NS/LAH (4%), respectively. None of the analyzed patients carried a mutation in the *KRAS* gene.

Familial cosegregation of the trait and mutation was documented in 8 families (14% of genotyped cases), including 4/20 NS cases and 1/11 LS cases with *PTPN11* mutations, 2/8 cases with *SOS1* mutations, and in the single case with *CBL* mutation. In 6 of the 8 families, the mutated allele was transmitted by the affected mother.

Clinical data were reviewed by analyzing available clinical records. Information was collected on pregnancy, delivery, growth parameters at birth, clinical features, including major and minor anomalies or defects depicted by cerebral ultrasound examination or MRI, 2-dimensional color-Doppler echocardiography, renal ultrasonography, audiological evaluation by BSERA, neurological assessment for hypotonia and developmental delay. Facial dysmorphism was considered to be marked, in the presence of 6 or more features, including hypertelorism, downslanting palpebral fissures, epicanthal folds, short broad nose, deeply grooved philtrum, high wide peaks of the lip vermilion, micrognathia, low-set and/or posteriorly angulated ears with thick helices, and low posterior hairline, while they were considered mild in the presence of 5 or less features [Allanson et al., 2010]. Clinical diagnosis before and after genetic testing was recorded.

Results

The clinical features recorded in the mutation-positive subjects with RASopathy are summarized in table 2. Mean age at the time of clinical diagnosis was 4.7 months. The comparison of clinical diagnosis before genetic test-

ing and after molecular diagnosis is reported in table 3. Mildly or markedly expressed facial anomalies were found in all patients (fig. 1). Newborns/infants with CS had coarse facial appearance. Additional clinical features invariably documented in each RASopathy included: (1) polyhydramnios and short neck/pterygium in CS; (2) weight below the <3rd centile in NS/LAH, CS, and *RAF1* mutation-positive NS; (3) thoracic anomalies in *BRAF* mutation-positive CFCS; (4) congenital heart defects (CHD) in NS and LS; (5) developmental anomalies in NS/LAH, CFCS, and CS; (6) feeding difficulties in patients with NS/LAH, and CS; (7) laryngomalacia in *RAF1* mutation-positive NS; (8) hair anomalies in patients with NS/LAH.

In table 4, the prevalence of clinical features in the present series is compared to percentages found in published reports with wider age distribution.

Occasional findings included: (1) cerebral anomalies (corpus callosum hypoplasia and cerebellar hypoplasia, Arnold-Chiari malformation); (2) ocular anomalies (coloboma, microphthalmia, iris heterochromia, cataract, and nystagmus) (table 2).

Discussion

The retrospective analysis of clinical features in patients with molecularly confirmed RASopathies, documented during the first year of life, suggests that some characteristics can be used as hints for suspecting a RASopathy, in general, during the first months. In addition, some clinical signs may lead to the suspect of individual disorders or, within a disorder, to individually affected disease genes. Nevertheless, this is a selected cohort, so that the frequency of clinical features may not be applied for general infants with a RASopathy, but specifically for those within the first year of life. Some of the cases included in this report initially received a clinical diagnosis which was changed after the results of genetic testing (table 3). This occurred more often in patients with LS, originally classified as having NS, and in patients with *BRAF* mutation, which were diagnosed as having an unspecified Noonan-like syndrome.

CHD was the clinical finding leading to medical attention in patients with NS and LS, being invariably present, independently of the affected disease gene and type of mutation. Patients with CFCS and CS displayed CHD in 75% of the cases. In these infants, major reasons for the patients' referral were either feeding difficulties (80–100%) or developmental motor delay (100%).

Table 1. Molecular characterization and clinical diagnosis of the patients included in the study

Gene	Exon	Nucleotide substitution	Amino acid substitution	Disorder	Number of patients
PTPN11	2	c.124A>G	T42A	NS	1
PTPN11	3	c.172A>G	N58D	NS	1
PTPN11	3	c.174C>G	N58K	NS	1
PTPN11	3	c.184T>G	Y62D	NS	4
PTPN11	3	c.188A>G	Y63C	NS	1
PTPN11	3	c.214G>T	A72S	NS	1
PTPN11	3	c.228G>T	E76D	NS	1
PTPN11	3	c.236A>G	Q79R	NS	1
PTPN11	7	c.836A>C	Y279S	LS	2
PTPN11	7	c.836A>C	Y279C	LS	1
PTPN11	8	c.922A>G	N308D	NS	4
PTPN11	8	c.923A>G	N308S	NS	3
PTPN11	12	c.1381G>A	A461T	LS	1
PTPN11	12	c.1403C>T	T468M	LS	4
PTPN11	13	c.1472C>T	P491L	NS	2
PTPN11	13	c.1492C>T	R498W	LS	1
PTPN11	13	c.1528C>G	Q510E	LS	2
SOS1	11	c.1301_2GG>AA	G434K	NS	1
SOS1	11	c.1649T>C	L550P	NS	1
SOS1	11	c.1654A>G	R552G	NS	3
SOS1	11	c.1656G>C	R552S	NS	1
SOS1	14	c.2104T>C	Y702H	NS	1
SOS1	15	c.2186G>T	W729L	NS	1
NRAS	3	c.179G>A	G60E	NS	1
RAF1	7	c.768G>T	R256S	NS	1
RAF1	7	c.770C>T	S257L	NS	2
RAF1	7	c.779C>G	T260R	NS	1
BRAF	6	c.722C>G	T241R	NS	1
BRAF	6	c.735A>T	L245F	CFCS	1
BRAF	11	c.1406G>A	G469E	CFCS	1
BRAF	12	c.1501G>A	E501K	CFCS	1
BRAF	15	c.1801A>C	K601Q	CFCS	1
HRAS	1	c.34G>A	G12S	CS	3
HRAS	1	c.38G>A	G13D	CS	1
SHOC2	1	c.4A>G	S2G	NS/LAH	2
MEK2	2	c.186_197del	K63_E66del	CFCS	1
CBL	9	c.1259G>A	R420Q	NS	1

CFCS = Cardiofaciocutaneous syndrome; CS = Costello syndrome; LS = LEOPARD syndrome; NS = Noonan syndrome; NS/LAH = Noonan-like syndrome with loose anagen hair.

Anatomic types of CHD were variable in the different disorders and molecular subgroups. Pulmonary valve stenosis was more common in newborns and infants with a diagnosis of NS associated with mutations in *PTPN11* and *SOS1*, and in CFCS subjects with *BRAF* mutation, while hypertrophic cardiomyopathy was significantly as-

Table 2. Clinical features in 57 patients with molecularly confirmed RASopathy

Gene phenotype	*PTPN11* NS	*PTPN11* LS	*SOS1* NS	*RAF1* NS	*SHOC2* NS/LAH	*NRAS* NS	*CBL* NS	*BRAF* NS	*BRAF* CFCS	*MEK2* CFCS	*HRAS* CS
Number of patients	20	11	8	4	2	1	1	1	4	1	4
Sex	14 m, 6 f	4 m, 7 f	5 m, 3 f	2 m, 2 f	1 m, 1 f	1 f	1 f	1 f	3 m, 1 f	1 m	4 m
Polyhydramnios	8/20 (40%)	5/11 (45%)	6/8 (75%)	3/4 (75%)	0	0	0	0	1/4 (25%)	0	4/4 (100%)
Prenatal/neonatal lymphatic anomalies	11/20 (55%)	3/11 (27%)	2/8 (25%)	0	0	0	0	0	2/4 (50%)	1	2/4 (50%)
High birth weight (>97th centile)	6/20 (30%)	4/11 (36%)	5/8 (63%)	1/4 (25%)	1/2 (50%)	1	0	1	2/4 (50%)	0	3/4 (75%)
Weight <3rd centile (at observation)	12/20 (60%)	7/11 (64%)	2/8 (25%)	4/4 (100%)	2/2 (100%)	0	0	1	2/4 (50%)	1	4/4 (100%)
Length <3rd centile (at observation)	14/20 (70%)	4/11 (36%)	2/8 (25%)	3/4 (75%)	2/2 (100%)	1	0	1	1/4 (25%)	1	4/4 (100%)
Median age at observation, months	5	3	6	1	5	2	6	9	5	2	2
Facial anomalies	20/20 (100%)	11/11 (100%)	8/8 (100%)	4/4 (100%)	2/2 (100%)	1	1	1	4/4 (100%)	1	4/4 (100%)
Marked facial phenotype	12/20 (60%)	4/11 (36%)	6/8 (75%)	2/4 (50%)	1/2 (50%)	1	1	0	3/4 (75%)	1	4/4 (100%)
Mild facial phenotype	8/20 (40%)	7/11 (64%)	2/8 (25%)	2/4 (50%)	1/2 (50%)	0	0	1	1/4 (25%)	0	0
Protruding tongue	5/20 (25%)	2/11 (18%)	7/8 (88%)	3/4 (75%)	2/2 (100%)	1	0	0	2/4 (50%)	0	4/4 (100%)
Short neck/pterygium	13/20 (65%)	4/11 (36%)	7/8 (88%)	3/4 (75%)	1/2 (50%)	1	1	0	3/4 (75%)	1	4/4 (100%)
Thorax anomalies	12/20 (60%)	8/11 (73%)	7/8 (88%)	3/4 (75%)	1/2 (50%)	1	0	1	4/4 (100%)	1	2/4 (50%)
Congenital heart defect	20/20 (100%)	11/11 (100%)	8/8 (100%)	4/4 (100%)	2/2 (100%)	1	1	1	3/4 (75%)	1	3/4 (75%)
Pulmonary valve stenosis	14/20 (70%)	3/11 (27%)	7/8 (88%)	1/4 (25%)	0	1	0	1	2/4 (50%)	1	1/4 (25%)
Atrial septal defect	3/20 (15%)	1/11 (9%)	3/8 (38%)	1/4 (25%)	1/2 (50%)	0	0	0	1/4 (25%)	0	0
Hypertrophic cardiomyopathy	0	9/11 (82%)	1/8 (13%)	4/4 (100%)	1/2 (50%)	1	0	0	2/4 (50%)	1	2/4 (50%)
Mitral valve anomaly	1/20 (5%)	3/11 (27%)	0	2/4 (50%)	1/2 (50%)	1	1	0	1/4 (25%)	0	1/4 (25%)
Atrioventricular canal	2/20 (10%)	0	0	0	0	0	0	0	0	0	0
Ventricular septal defect	2/20 (10%)	0	1/8 (13%)	0	0	0	0	0	0	0	0
Aortic coarctation	1/20 (5%)	0	0	0	0	0	0	0	0	0	0
Tetralogy of Fallot	0	0	0	1/4 (25%)	0	0	0	0	0	0	0
Renal anomaly	3/20 (15%)	2/11 (18%)	0	2/4 (50%)	0	0	0	0	0	0	0
Cryptorchidism (males)	11/14 (79%)	1/4 (25%)	4/5 (80%)	1/2 (50%)	0	n.a.	n.a.	n.a.	2/3 (67%)	1	2/4 (50%)
Developmental anomalies	10/20 (50%)	5/10 (50%)	1/8 (13%)	2/4 (50%)	2/2 (100%)	1	1	1	4/4 (100%)	1	4/4 (100%)
Mild motor delay	8/20 (40%)	4/10 (40%)	1/8 (13%)	1/4 (25%)	1/2 (50%)	0	1	1	1/4 (25%)	0	0
Moderate/severe motor delay	2/20 (10%)	1/10 (10%)	0	1/4 (25%)	1/2 (50%)	1	0	0	3/4 (75%)	1	4/4 (100%)
Epilepsy	1/20 (5%)	0	0	0	1/2 (50%)	0	0	1	1/4 (25%)	0	1/4 (25%)
Cerebral anomalies	0	0	0	0	0	0	1	0	1/4 (25%)	0	1/4 (25%)
Ocular anomalies	0	3/11 (27%)	0	0	0	0	0	0	3/4 (75%)	1	2/4 (50%)
Retinal coloboma	0	1/11 (9%)	0	0	0	0	0	0	1/4 (25%)	0	0
Microphthalmia (unilateral)	0	1/11 (9%)	0	0	0	0	0	0	0	0	0
Iris heterochromia	0	1/11 (9%)	0	0	0	0	0	0	0	0	0
Nystagmus	0	0	0	0	0	0	0	0	2/4 (50%)	0	1
Cataract	0	0	0	0	0	0	0	0	0	1	1
Deafness	0	1/11 (9%)	0	0	0	0	0	0	0	0	0
Feeding difficulties	13/20 (65%)	6/11 (55%)	0	3/4 (75%)	2/2 (100%)	0	0	1	3/4 (75%)	1	4/4 (100%)
Laryngomalacia	0	0	0	4/4 (100%)	0	0	0	0	1/4 (25%)	1	2/4 (50%)
Hair features	2/20 (10%)	3/11 (27%)	2/8 (25%)	1/4 (25%)	2/2 (100%)	1	1	0	3/4 (75%)	1	2/4 (50%)
Curly hair	0	3/11 (27%)	0	0	0	0	0	0	0	0	0
Thin/absent hair	2/20 (10%)	0	2/8 (25%)	1/4 (25%)	2/2 (100%)	1	1	0	3/4 (75%)	1	2/4 (50%)
Skin features	3/20 (15%)	9/11 (82%)	4/8 (50%)	1/4 (25%)	2/2 (100%)	0	1	0	4/4 (100%)	1	1/4 (25%)
Keratosis pilaris	0	0	4/8 (50%)	0	1/2 (50%)	0	1	0	4/4 (100%)	1	0
Eczema	0	0	2/8 (25%)	0	2/2 (100%)	0	0	0	2/4 (50%)	1	1/4 (25%)
Café-au-lait spot	3/20 (15%)	9/11 (82%)	0	1/4 (25%)	0	0	1	0	2/4 (50%)	0	0
Lentigines	0	2/11 (18%)	0	0	0	0	0	0	0	0	0
Familial segregation	4/20 (20%)	1/11 (9%)	2/8 (25%)	0	0	0	1	0	0	0	0

CFCS = Cardiofaciocutaneous syndrome; CS = Costello syndrome; LS = LEOPARD syndrome; NS = Noonan syndrome; NS/LAH = Noonan-like syndrome with loose anagen hair.

Table 3. Comparison of clinical diagnoses before and after genetic testing

Mutated gene	Pre-test clinical diagnosis	Post-test clinical diagnosis	Patients
PTPN11	NS	NS	20
	NS	LS	7
	LS	LS	4
SOS1	NS	NS	8
RAF1	NS	NS	4
SHOC2	NS	NS/LAH	1
	NS-like	NS/LAH	1
NRAS	NS	NS	1
CBL	NS	NS	1
BRAF	NS-like	CFCS	3
	CFCS	CFCS	1
	NS	NS	1
MEK2	CFCS	CFCS	1
HRAS	Costello	Costello	3
	NS-like	Costello	1

CFCS = Cardiofaciocutaneous syndrome; NS = Noonan syndrome; LS = LEOPARD syndrome; NS/LAH = Noonan-like syndrome with loose anagen hair; NS-like = Noonan-like syndrome.

sociated with LS-causing *PTPN11* mutations and NS-causing *RAF1* mutations. Such associations are in line with previously observed genotype-phenotype correlations [Tartaglia et al., 2002; Sarkozy et al., 2003; Digilio et al., 2006b, 2009; Limongelli et al., 2007; Pandit et al., 2007]. Of note, a wide spectrum of CHDs was documented in the neonatal series, including also atrioventricular canal defect, mitral anomalies, and aortic coarctation. The prevalence of CHDs in this report was slightly higher compared to published series comprising patients with a wider age distribution (table 4). This could be due to the fact that CHDs are often the first clinical symptoms attracting medical attention and suspicion of a RASopathy in the neonatal period and during infancy. On the other hand, the finding that all patients with NS displayed CHDs and facial anomalies actually means that the presence of a heart defect and some facial features suggestive of NS were necessary for making an early diagnosis of NS, while an early diagnosis can be missed in NS patients without a heart defect.

Facial features were present in all patients, being either mildly or markedly expressed. We were unable to identify a distinct pattern suggestive for any molecular subtype or disorder, with the only exception of the occurrence of 'coarse' appearance in infants with CS. This finding is in agreement with the conclusions of a recently published study [Allanson et al., 2010]. Protruding tongue was found in all patients with NS/LAH and CS, and in a large proportion of NS newborns and infants with a *SOS1* mutation.

Feeding problems caused by failure to thrive were highly common in patients with CFCS, CS, and NS/LAH. They consisted of poor suck and slow feeding with recurrent vomiting, requiring tube feeding or gastrostomy during the first year of life. The degree of growth deficiency appears related to severity of the feeding problems and CHD, in particular in the presence of hypertrophic cardiomyopathy.

Delayed developmental milestones and hypotonia were found in approximately half of mutation-positive patients, and, in general, developmental delay was milder in babies with *PTPN11* mutations, and more severe in those with CFCS and CS. Length of hospitalization and the degree of poor growth were likely related to delayed motor development, but a possible relationship with the molecular defect cannot be excluded. This is consistent with previous surveys in older patients pointing to a more severe impairment in the presence of mutations affecting the downstream components of the RAS-MAPK pathway [Cesarini et al., 2009; Pierpont et al., 2009]. Discordance in prevalence of delayed developmental milestones in different ages is found in patients with LS associated with *PTPN11* mutations (table 4), with a lower occurrence in older patients.

Epilepsy was found in 40% of infants with CFCS associated with *BRAF* mutations, in 25% of CS individuals, and only in one case of NS due to *PTPN11* mutation.

Among ocular defects, coloboma and microphthalmia were seen in patients with *PTPN11* mutations, while cataract, nystagmus and visus defects occurred in CFCS patients with *BRAF* and *MEK2* mutations and in patients with Costello syndrome.

Laryngomalacia was present in patients with *RAF1* and *HRAS* gene mutations.

Ectodermal and skin features are hallmarks for differentiating RASopathies during childhood and adolescence (table 4). Consistent with the distinctive hair anomalies (i.e., easily pluckable, sparse, thin, slow-growing hair characterized by an anagen stage of hair follicle development and bulbs lacking internal and external root sheats) associated with the p.Ser2Gly *SHOC2* substitution [Mazzanti et al., 2003; Cordeddu et al., 2009], abnormal hair features compatible with a loose anagen hair condition was invariantly observed during the first year

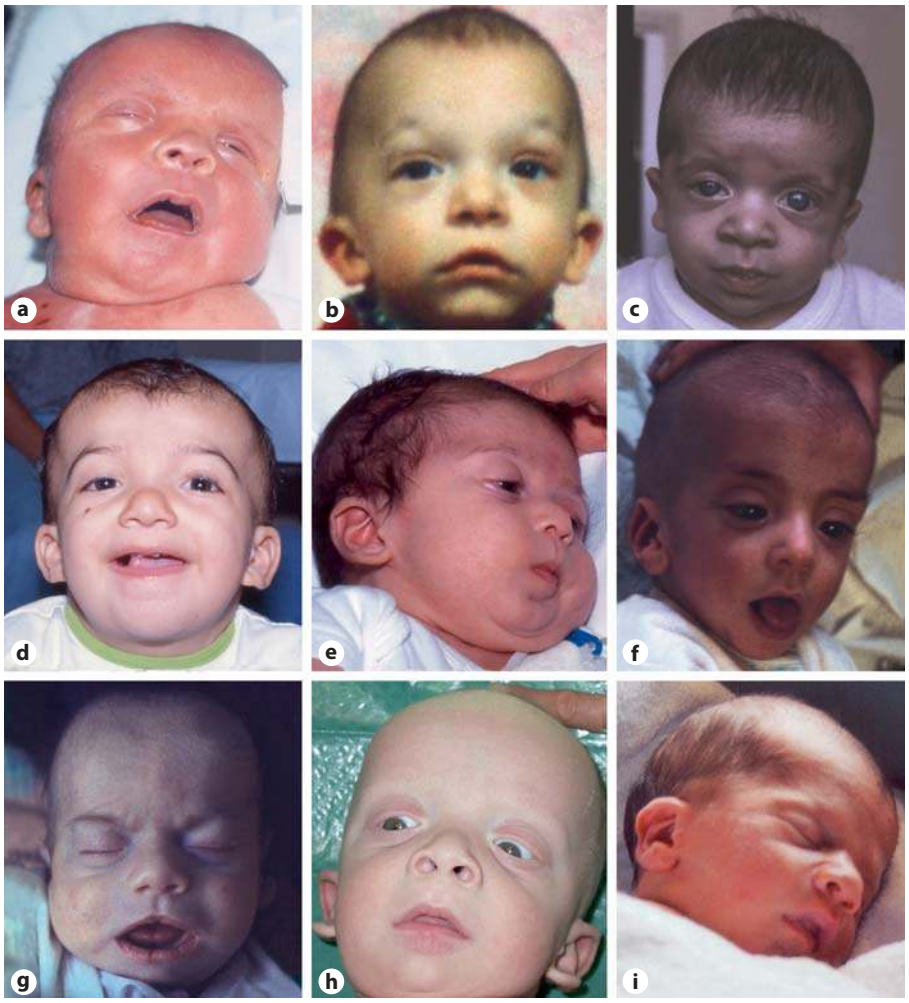

Fig. 1. Faces of infants with RASopathies. The panels show representative facial features occurring in newborns and infants with Noonan syndrome caused by *PTPN11, RAF1, SOS1* and *NRAS* mutations (**a–d**), LEOPARD syndrome due to a *PTPN11* mutation (**e**), Noonan-like syndrome with loose anagen hair resulting from the c.A>G change in *SHOC2* (**f**), cardiofaciocutaneous syndrome due to *BRAF* (**g**) and *MEK2* (**h**) mutations, and Costello syndrome caused by the c.34G>A *HRAS* mutation (**i**).

of life in patients with the invariant *SHOC2* mutation. Of note, thin, sparse and/or wispy hair occurred preferentially in association with *NRAS*, *BRAF*, *MEK1*, and *HRAS* mutations, while normal hair was generally observed in association with *PTPN11*, *SOS1* and *RAF1* mutations. Hair was often lacking in the first year of life in patients with CFCS caused by *BRAF* or *MEK2* mutations, in which an eczematous skull skin was also present (table 4). These findings indicate that hair characteristics might significantly change with time, as in the case of CS, which is characterized by the distinctive curly and kinky hair structure observed during childhood and adulthood.

Lentigines and café-au-lait spots were found in infants with LS-causing *PTPN11* mutations, while keratosis pilaris over the face and the extensor surfaces and eczema were documented to be more common in those with *SOS1*, *SHOC2* and *BRAF* mutations. Generalized neonatal ichthyosis was found in single patients with *SHOC2* and *MEK2* mutations. Of note, all these skin features were present in the first months of life, with the only exception of lentigines, which developed in 9 of the 11 cases with molecularly confirmed LS after the second year of life (table 4) [Digilio et al., 2006a].

In conclusion, analysis of clinical features in the present series of patients with RASopathies assessed during the first year of life suggests that the following characteristics can be useful for early prediction of the molecular subtype of the disorder: (1) anatomic type of CHD, (2) severity of feeding difficulties and developmental milestones, (3) hair and skin anomalies. In contrast, facial features are useful in making the general diagnosis of RASopathy, but are not helpful in determining the specific molecular subtype. While larger co-

Table 4. Comparison of the prevalence (%) of clinical features in the present newborn/infant series with data of published reports with wider age distribution

Disorder-disease gene:	NS-*PTPN11*		LS-*PTPN11*		NS-*SOS1*		NS-*RAF1*		NS/LAH-*SHOC2*		CFCS-*BRAF*		CS-*HRAS*	
Reference no. in footnote:	1	2	1	3	1	4	1	5	1	6	1	7	1	8
Facial anomalies	100	85	100	87	100	100	100	100	100	100	100	100	100	100
Short neck/pterygium	65	52	36	28	88	75	75	83	50	68	75	65	100	100
Thorax anomalies	60	65	73	70	88	82	75	71	50	80	100	63	50	72
Congenital heart defect	100	83	100	71	100	86	100	93	100	80	75	86	75	63
Developmental retardation	50	41	50	20	13	15	50	55	100	83	100	100	100	100
Epilepsy	5	10	0	0	0	3	0	n.r.	50	n.r.	25	17	25	20
Short stature	70	76	36	25	25	36	75	90	100	100	25	80	100	96
Deafness	0	rare	9	20	0	0	0	n.r.	0	n.r.	0	25	0	n.r.
Feeding difficulties	65	63	55	n.r.	0	23	75	n.r.	100	n.r.	75	95	100	100
Laryngomalacia	0	n.r.	0	n.r.	0	n.r.	100	n.r.	0	n.r.	25	n.r.	50	n.r.
Hair anomalies	10	34	27	54	25	80	25	22	100	100	75	95	50	80
Keratosis pilaris	0	6	0	0	50	43	0	10	50	24	100	80	0	62
Café-au-lait spot	15	10	82	75	0	0	25	30	0	n.r.	50	20	0	n.r.
Lentigines	0	0	18	92	0	0	0	n.r.	0	n.r.	0	14	0	10

CFCS = Cardiofaciocutaneous syndrome; CS = Costello syndrome; LS = LEOPARD syndrome; n.r. = not reported; NS = Noonan syndrome; NS/LAH = Noonan-like syndrome with loose anagen hair.

1 = present series. 2 = Tartaglia et al. [2002]; Sarkozy et al. [2003]; Zenker et al. [2004]. 3 = Digilio et al. [2002]; Legius et al. [2002]; Sarkozy et al. [2009a]. 4 = Roberts et al. [2007]; Tartaglia et al. [2007]; Zenker et al. [2007]. 5 = Pandit et al. [2007]; Razzaque et al. [2007]. 6 = Cordeddu et al. [2009]. 7 = Rodriguez-Viciana et al. [2006]; Nava et al. [2007]; Sarkozy et al. [2009a]. 8 = Hennekam [2003]; Kerr et al. [2006]; Zampino et al. [2007].

horts are required to attain a more accurate clinical definition of each condition during the neonatal period and infancy, the documented correlations denote that a subset of features can direct towards a prompt diagnosis, and a more effective patient management and genetic counseling.

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