RASopathies: Evolving Concepts in Pathogenetics, Clinical Features, and Management

Abstract

RASopathies refers to the group of disorders which are caused by a mutation in various genes of the RAS/MAPK (RAT sarcoma virus/Mitogen activated protein kinase) pathway. It includes many genes with varied functions, which are responsible for cell cycle regulation. As the mutation in one gene affects the entire pathway, there are many overlapping features among the various syndromes which are included under an umbrella term "RASopathies." However, neuroectodermal involvement is a unifying feature among these syndromes, which are caused by germline mutations affecting genes along this pathway. Recently, many other RASopathies have been described to involve blood vessels, lymphatics, and immune system. Also, many cutaneous mosaic disorders have been found to have mutations in the concerned pathway. The purpose of this article is to briefly review the pathogenesis of RASopathies with cutaneous manifestations, and summarise the features that can be helpful as diagnostic clues to dermatologists. As we understand more about the pathogenesis of the pathway at the cellular level, the research on genotype-phenotype correlation and therapeutic options broadens. Targeted therapy is in the clinical and preclinical trial phase, which may brighten the future of many patients.

Keywords: *Newer drugs, pathogenetics, RASopathies*

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Introduction

RAt Sarcoma (*RAS*) genes share sequence homology with Harvey (HRAS) and Kristen (KRAS) rat sarcoma viruses.[1] RAS/MAPK (Mitogen activated protein kinase) pathway has an important role in the regulation of the cell cycle. Previously the term "RASopathies" was used to describe germline mutations associated with multisystem developmental anomaly syndromes with predispositions to various neoplasms, but now the terminology has been broadened to include diseases with somatic mutations associated with increased risk of cancers[2] and multigenic diseases as well.[3] Recently, mutations along the concerned pathway have been shown in many mosaic cutaneous disorders, vascular malformation syndromes, and primary immune deficiency.

RAS/MAPK proteins are encoded by various genes located on different chromosomes. Hence, there are some unique features in each syndrome caused by germline mutations apart from unifying neuroectodermal involvement. Classification and definition of RASopathies are not standardized yet, but diseases and malignancies described to have mutations in the concerned pathway are listed in Table 1. Many cancers have mutations in *RAS* genes; *KRAS* mutations are most frequent, followed by *NRAS* and *HRAS*.[4] The most common cutaneous malignant tumor to have a mutation in the RAS/MAPK pathway is malignant melanoma. However, it is also necessary to target other pathways important in the development and progress of melanoma.[1] This article will further concentrate on syndromic diseases with cutaneous manifestations only.

Pathogenetics and evolving concepts

RAS/MAPK pathway has a crucial role in the regulation of the cell cycle [Figure 1]. Mutations involving one gene will affect the entire pathway. Most RASopathies are caused by activating mutations affecting various genes, which result in overactivity of MAPK/MEK which will be responsible for increased signaling. The only exception to this is Noonan syndrome with multiple lentigines (NS-ML) which is caused by

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Table 1: Germline and somatic mutations in RAS/MAPK pathway, related conditions, and genes affected[1,2,5-8]

Type of mutation	Disease/syndrome	Genes affected
Germline mutation	Noonan syndrome	*PTPN11, SOS1, SOS2, RAF1, BRAF, KRAS, NRAS, RIT1, LZTR1*
	Noonan syndrome with multiple lentigines/LEOPARD syndrome	*PTPN11, RAF1, BRAF, NRAS*
	Noonan syndrome with loose anagen hair syndrome	*SHOC2*
	Noonan-neurofibromatosis 1 syndrome	*NF-1/PTPN11*
	Neurofibromatosis type 1	*NF1*
	Legius syndrome	*SPRED1*
	Costello syndrome	*HRAS*
	Cardiofaciocutaneous syndrome	*BRAF, KRAS, MAP2K1, MAP2K2*
	Central conducting lymphatic anomaly syndromes	*ARAF, KRAS*
	SYNGAP 1 syndrome	*SYNGAP 1*
	Capillary malformation-arteriovenous malformation syndrome	*RASA1*
	Type 1 Hereditary gingival fibromatosis	*SOS1*
Somatic mutations in mosaic disorders	Keratinocytic epidermal nevus	*HRAS, KRAS, NRAS, FGFR3, PIK3CA*
	Nevus sebaceous, Schimmelpenning-Feuerstein-Mims syndrome, neuroblastoma, pancreatic, lung, and colorectal cancers, tumours of the adrenal gland, brain, kidney, bones, breast, cervix, etc.	*HRAS, KRAS, NRAS*
	Phacomatosis pigmentokeratotica	*HRAS*
	Phacomatosis pigmentovascularis	*GNAQ, GNA11, GNA14*-increases MAPK activity
	Melanocytic nevi	*BRAF*
	Nevus spilus	*HRAS*
	Nevus spilus like congenital melanocytic nevi	*NRAS*
	Agminated segmental melanocytic nevi	*HRAS, BRAF*
	Sporadic or agminated spitz nevi	*HRAS*
	Cutaneous skeletal hypophosphatemia syndrome, childhood lobular capillary haemangioma, GLUT-1 negative non-involuting congenital hemangioma	*RAS*
Somatic mutations other disorders and tumors	RAS-associated autoimmune leukoproliferative disorder (RALD)	*KRAS, NRAS*
	Autoimmune lymphoproliferative syndrome (ALPS)	*NRAS*
	Juvenile myelo-monocytic leukemia (JMML)	*NRAS, KRAS, PTPN11, CBL, NF1* (could be seen in germline mutation of same genes associated syndrome)
	Several types of histiocytosis	*NRAS, KRAS, MAP2K1, MAP2k2, ARAF, PTPN11*
	Hairy cell leukemia	*BRAF*
	Hodgkin's lymphoma	*RASGRP1*
	Melanoma	*BRAF, NRAS*

inactivating mutations, and the exact mechanisms by which activating and inactivating mutations result in similar phenotype are not well-understood.[1]

Emerging role of new genes in RASopathies

Next-generation sequencing (NGS) has found a few other genes that were altered in patients of RASopathies, but functionally they have been not validated yet. *KAT6B, RREB1,* and *CDC42* alterations have been found to be associated with NS-like features, while a mutation in *YWHAZ* has been associated with cardio-facio-cutaneous syndrome (CFCS).[5]

Senescence in RASopathies

Senescence basically refers to permanent cell cycle arrest. This phenomenon is responsible for tumor suppression, embryonic development, adult tissue remodeling, and physiological aging.[9] RAS/MAPK pathway is crucial for this phenomenon, and inhibition of MEK is associated with longevity and improved cellular health in animal models. It has been postulated that senescence is the possible mechanism that may be responsible for the premature aging in syndromic RASopathies.[10,11] This was observed in animal models with *HRAS* mutations, which manifested as reduced cellular proliferation, papillomatous growth with senescence cells, and reduced life span.[9] The same features were observed in one study that patients with Costello syndrome (CS) exhibit premature-aged skin appearance, hair loss, hypotonia, osteopenia, and bladder cancer at a young age.[12,13]

RAS/MAPK pathway and immune system

RAS/MAPK pathway, PI3K-AKT-mTOR pathway, and RalGDS pathway are closely related to each other, and all

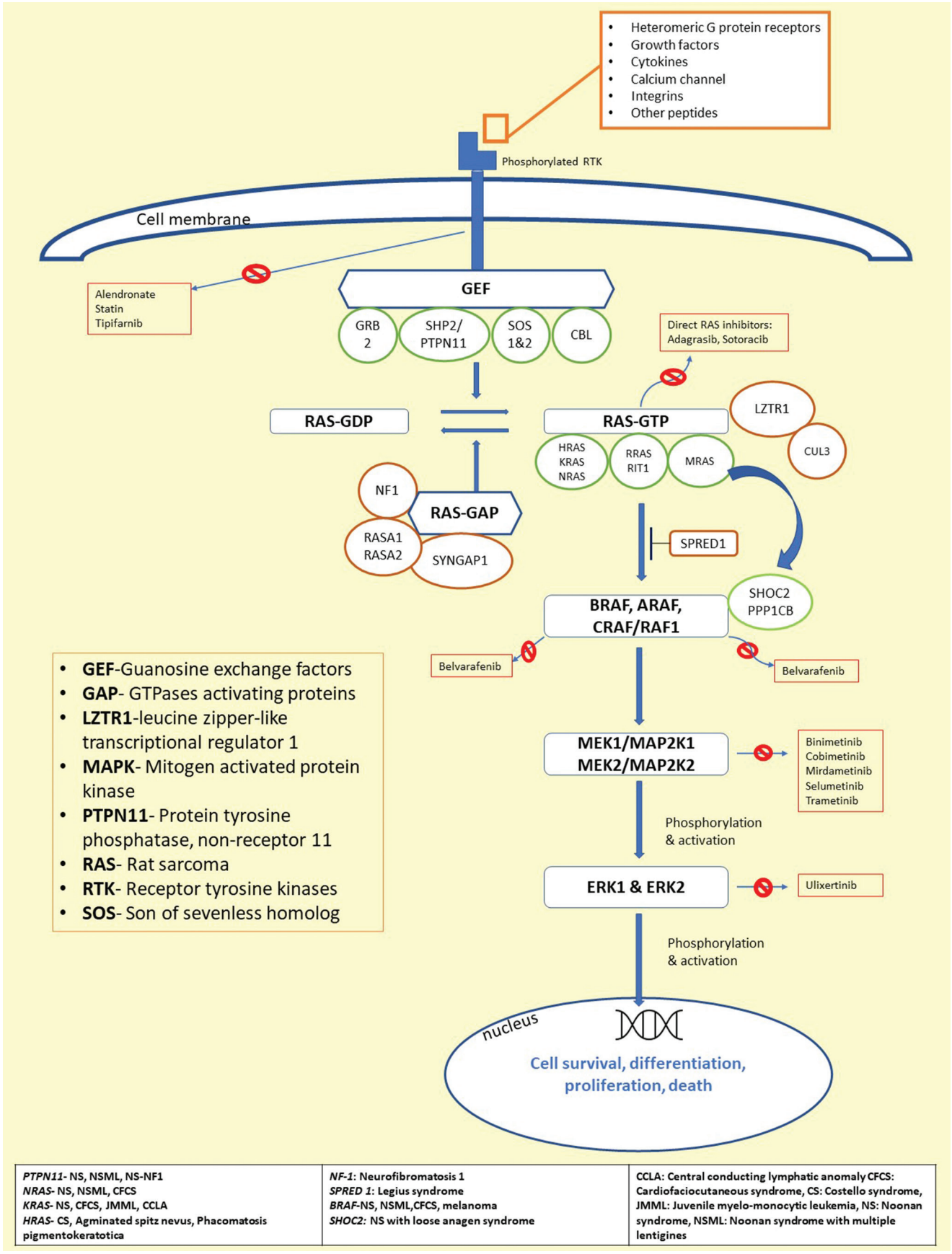

Figure 1: RAS/MAPK pathway. Genes circled in brown are negative modulators of the pathway. Various activating factor when binds to receptor tyrosine kinases the cascade of the pathway is activated which in turn are responsible for regulation of cell cycle. Therapeutic targets and relevant drugs are mentioned in red square box

three play important roles in adaptive immunity.[14] One study evaluated patients of RASopathies and found that though there are no clinical signs of immune deficiency in these subsets, they have some delay in B cell maturations, which would have contributed towards tonsillar and adenoid hypertrophy. Also, recently, an immune-deficiency syndrome with mutation in *RASGRP1* has been described in which patients develop combined immune deficiency with susceptibility to various viral, fungal, and bacterial infections as well as Epstein Barr virus-associated lymphoproliferative disorders and autoimmune cytopenias.[15]

RAS-associated autoimmune leukoproliferative disorder (RALD) has been described, which shares features with autoimmune lymphoproliferative syndrome. Also, there have been reports of autoimmune disorders like systemic lupus erythematosus, Hashimoto thyroiditis, celiac disease, etc., in patients with RASopathies. One retrograde study had an interesting finding in terms of low IgA levels in patients with Noonan Syndrome (NS), NS-ML, and CFCS.[16] Clinically low IgA can be associated with recurrent infections involving upper respiratory tract and gastrointestinal tract, as well as autoimmunity and allergies. The same study also reported a high titre of thyroid autoantibodies in euthyroid patients, and hence suggested that patients can be monitored in the future for the development of thyroid diseases. They also suggested a hypothesis that impairment along the concerned pathway could result in low CD8 production and high IL4 level, which may be responsible for induction of autoimmunity.

RAS/MAPK pathway, hematopoietic malignancy, and histiocytosis

All cases of NF1 associated juvenile myelo-monocytic leukemia (JMML) have been associated with secondary additional mutations which are not seen in cases of JMML developing in the setting of NS. So, secondary events may be responsible for the evolution of RALD to JMML.[3] It is a matter of further research that why some patients develop RALD or histiocytosis with or without autoimmunity.

Clinical features

Phenotypic overlap is characterized by similar symptoms in different diseases, and it usually suggests genotypic overlap.[17] Overall, the Noonan-phenotype is the most common and most other RASopathies have overlapping features with NS [CALM (Café-au-lait macules), ectodermal anomalies, Noonan-like facies, cardiac and musculoskeletal involvement, etc.]; though all have characteristic unique features which can be suggestive clinically. Cutaneous features and other manifestations are dynamic in these syndromes and manifestations vary according to age.[6] Cutaneous,[1,2,7,18-20] extracutaneous manifestations,[1,2,7,18-20] and diagnostic criteria[21-23] for various RASopathies are summarized in Table 2, Table 3, and Table 4, respectively. More studies are required for phenotype and genotype correlation.

Noonan syndrome (NS)

The most common mutated gene in NS is *PTPN11* followed by *SOS1*. Classical phenotypic characteristics of this syndrome include facial dysmorphism, congenital heart defects (CHD), short stature, coagulopathies, and mental retardation. Patients usually present with systemic symptoms like feeding difficulties, vomiting, cognitive dysfunction, language dysfunction, bleeding tendency, and easy bruising.

Facial dysmorphism is a dynamic feature of this syndrome, and is less pronounced as the patient ages. A new-born will have the appearance of macrocephaly because of the large forehead and narrow temples along with other findings like hypertelorism, blue iris, downward slant of eyes, epicanthal folds, arched eyebrows, deeply grooved philtrum, upturned lips, gingival exposure on smiling, macroglossia, short neck, pointed chin, and low set posteriorly rotated ears. As the child grows, the facial features sharpen, and wispy-curly hair and webbing of the neck are visible. In adulthood, very few features like posteriorly rotated ears, webbing of neck, arched eyebrow, hypertelorism, pointed chin, prominent nasolabial folds, and recessed frontal hairline are visible, which may make diagnosis more difficult.

Although no genotype-phenotype correlation has been documented, a few features are associated with specific gene involvement. Mutation in *PTPN11* is associated with pulmonary stenosis (PS), atrial septal defects (ASD), short stature, coagulopathies, chest deformities, and keratinization disorders,[24,25] while a mutation in *SOS1* is associated with PS, ectodermal disorders, hypertelorism, and ptosis.[26] NS due to *KRAS* mutations shares features with CS and CFCS. Mutations in *RAF1* are characterized by hypertrophic cardiomyopathy (HOCM), significant intellectual disability,[27] laryngomalacia, hoarse cry,[7] and pigmented lesions, while patients with mutations in *BRAF* have melanocytic nevi and lentigines. Patients having mutations in *CBL* have a higher risk of myeloproliferative disorders. Refractive errors and optic nerve abnormalities are more common in mutations of *RAF1* and *KRAS*.

Noonan syndrome with multiple lentigines (NS-ML)/ LEOPARD syndrome

LEOPARD syndrome is an old acronym[7] (Lentigines, Electrocardiographic abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retarded growth, and Deafness). Most of the time it is caused by inactivating mutations in *PTPN11,* and less commonly by activating mutations in *RAF1*. In comparison to NS, features of NS-ML accentuate as the patient ages. The characteristic dark brown lentigines appear at the age of 4–5 years, which increase to more than 1000 in number by pubertal age to involve mainly the face and upper trunk.[1] Lentigines do not affect the mucosa,[1] but may involve other parts like

Table 2: Cutaneous features of RASopathies[1,2,5,7,17,18]

RASopathies/prevalence	Characteristic cutaneous features	Other findings
Noonan syndrome (1:1000-2500)	Ectodermal anomalies (more common with PTPN11 and SOS1 mutations) • Keratosis pilaris • Ulerythema ophryogenes • Sparse scalp hair, sparse/absent eyelashes Pigmented lesions (more common with RAF1 and BRAF mutations) • Lentigines • Melanocytic nevi Café-au-lait macules >3 (in PTPN11 mutations)	Lymphatic abnormalities Granular cell tumors Capillary malformation
LEOPARD syndrome/ NSML (1:3500)	Lentigines (absent in patients with NRAS muations) Café noir >3	
NS LAH (<1:1000000)	Thin, curly, sparse slow growing hair Easy pluckability Sparse/absent eyelashes	Temporal alopecia Keratosis pilaris
NF 1 (1:2500-3000)	Café-au-lait macules with a smooth border Crowe's sign (Axillary freckling) Neurofibroma (appears at adolescence/puberty/pregnancy) Plexiform neurofibroma (congenital)	Patrick Yesudian sign (Palmar freckling)
Legius syndrome (1:46,000-75000)	Café-au-lait macules Crowe's sign (axillary freckling)	Lipomas
Costello syndrome (1 in 1,290,000)	Cutaneous papilloma (72%) • Warty papules around alae nasi, anterior nares and peri anal area appear any time up to adolescence • May coalesce Loose redundant skin in hands and feet Bushy eyebrows Acanthosis nigricans Dark complexion	Diffuse palmoplantar keratoderma Pachydermatoglyphia Spatula-like finger pad Koilonychia Fast growing nails Slowly growing scalp hair, frontal balding Nevi and lentigines <50
CFCS (1 in 810,000)	Ectodermal anomalies • Absent scalp hair with scalp eczema at birth→scarce short, curly hair growth • Ulerythema ophyrogenes • Follicular hyperkeratosis • Scarring alopecia of eyebrows Palmoplantar keratoderma >50 melanocytic nevi	Haemangioma Fast growing nails Scarce body hair Folded earlobes Acanthosis nigricans Hyperplastic nipples Lymphedema
CM-AVM syndrome (1:100,000 in northern Europeans)	Small and large capillary malformations with surrounding white halo AVM involving skin and subcutaneous tissue	Capillary malformations involving vermilion border Lymphedema
Hereditary gingival fibromatosis (1/175,000)	Benign fibrous growth of gums	-

axillary/inguinal folds, palms and soles. However, the patient may have "café noir" which may be present before lentigines. Histopathologically,[28] it corresponds to either simple lentigines or melanocytic nevi. Ocular hypertelorism is seen in 100% of cases,[20] and almost all patients have a wide nasal bridge, palpebral ptosis, low set ears, premature facial wrinkling, and accentuated philtrum. Patients with *NRAS* mutations lack lentigines, ocular, cardiac, and hearing abnormalities.[25]

Noonan syndrome with loose anagen hair syndrome (NS-LAH)

Mutation in *SHOC2* results in this syndrome, which has overlapping signs with NS, but also has characteristically easy pluckability of hair[29] due to the absence of external and internal hair root sheath. This finding improves with age, and its diagnostic value declines. Apart from characteristic features[30,31] mentioned in Table 2, other features reported are low birth weight and feeding difficulties.

Table 3: Common extracutaneous manifestations of RASopathies[1,2,5,7,17,18]

RASopathies	Cardiac manifestations	Skeletal manifestations	Genitourinary manifestations	Malignancy risk
Noonan syndrome	Pulmonary valve stenosis Hypertrophic cardiomyopathy Atrial/ventricular septal defects Ostium secundum Peripheral pulmonary artery stenosis	Pectus carinatum Pectus excavatum Scapula alata Cubitus and genu valgus Joint hyperextensibility Clinobrachydactyly Scoliosis	Cryptorchidism Hyperplastic ovaries Pyelo-ureteric stenosis Hydronephrosis	4% risk Myeloproliferative disorders Juvenile myelo-monocytic leukemia Glioma Neuroblastoma Rhabdomyosarcoma
LEOPARD syndrome	Progressive myocardial hypertrophy Pulmonary valve stenosis Conduction disorders Repolarization abnormalities Abnormal QRS complex	Similar to Noonan syndrome	Cryptorchidism Hypospadias Genital hypoplasia Delayed onset puberty	Acute myeloid leukemia Acute lymphoblastic leukemia Myeloproliferative disorders Neuroblastoma Choristomas Melanoma
NF 1	Pulmonary stenosis Pulmonary hypertension	Short stature Osseous dysplasia (tibia, sphenoid) Bowing of tibia Pseudoarthrosis Scoliosis Cortical thinning of long bones Pectus excavatum Poly/syndactyly Genu valgus/varus	Hydronephrosis Double ureter	Pediatric malignancies Optic glioma Rhabdomyosarcoma Neuroblastoma Juvenile myelo-monocytic leukemia Adult malignancies Peripheral nerve sheath tumors Pheochromocytomas Breast cancer Stromal tumours
Costello syndrome	Hypertrophic cardiomyopathy (41%) Congenital heart defects (21%) Supraventricular tachycardia	Cubital deviation of wrist and thumb Retraction of heel Kyphoscoliosis Osteopenia Hyperflexible joints Scoliosis		Pediatric malignancies Rhabdomyosarcoma Neuroblastoma Adult malignancies Transitional cell carcinoma of the bladder
CFCS	Pulmonary stenosis Atrial septal defects Hypertrophic cardiomyopathy	Short stature Hypoplastic supraorbital ridges		Rhabdomyosarcoma Hepatoblastoma Lymphoblastic leukemia Non-Hodgkin's lymphoma
CM-AVM	Tetralogy of Fallot Septal and valvular defects Congestive heart failure	AVM involving bones		May be alike NF 1

Neurofibromatosis type 1 (NF 1)/Von Recklinghausen disease

NF 1 [Figure 2a] is the commonest of all RASopathies, with the estimated incidence being 1 in 2600-3000.[2] It is inherited as an autosomal dominant pattern or can occur because of spontaneous de novo mutations in the *NF1* gene located at 17q11.2. Neurofibromin is a negative regulator[1] of the RAS/MAPK pathway. So, mutations in it are associated with an increase in activity of the same pathway. Clinically it can be divided into three phenotypes as follows:[7]

- Mild phenotype (3-bp microdeletions in *NF1*)—Café-au-lait macules (CALM), few/absent neurofibroma, plexiform neurofibroma, optic pathway glioma, significant cognitive impairment.
- Noonan type (missense mutation of *Arg*)—CALM, Lisch nodule, PS, postnatal developmental delay.
- Severe phenotype (large deletions in full *NF1* and flanking genes)—early onset neurofibroma, spinal neurofibroma, dysmorphic face, tall for age, large hands and feet, hyperflexible joints, hypotonia, low

Table 4: Diagnostic criteria for RASopathies[21-23]

RASopathy	Major criteria
Neurofibromatosis type 1 (At least one pigmentary abnormality should be bilateral) (In presence of ipsilateral orbital plexiform neurofibroma, sphenoid dysplasia should not be considered a separate criterion)	A: The diagnostic criteria for NF1 are met in an individual who does not have a parent diagnosed with NF1 if two or more of the following are present: • Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals • Freckling in the axillary or inguinal region • Two or more neurofibromas of any type or one plexiform neurofibroma • Optic pathway glioma • Two or more iris Lisch nodules identified by slit lamp examination or two or more choroidal abnormalities (CAs)—defined as bright, patchy nodules imaged by optical coherence tomography (OCT)/near-infrared reflectance (NIR) imaging • A distinctive osseous lesion such as sphenoid dysplasia, anterolateral bowing of the tibia, or pseudarthrosis of a long bone • A heterozygous pathogenic NF1 variant with a variant allele fraction of 50% in apparently normal tissue such as white blood cells B: A child of a parent who meets the diagnostic criteria specified in 'A' merits a diagnosis of NF1 if one or more of the criteria in 'A' are present
Legius syndrome (Diagnosis can't be excluded if less than six café-au-lait macules are present)	A: The diagnostic criteria for Legius syndrome are met in an individual who does not have a parent diagnosed with Legius syndrome if the following CRITERIA are present: • Six or more café-au-lait macules bilaterally distributed and no other NF1-related diagnostic criteria except for axillary or inguinal freckling • A heterozygous pathogenic variant in SPRED1 with a variant allele fraction of 50% in apparently normal tissue such as white blood cells B: A child of a parent who meets the diagnostic criteria specified in 'A' merits a diagnosis of Legius syndrome if one or more of the criteria in 'A' are present
Mosaic neurofibromatosis type 1	The diagnostic criteria for mosaic NF1 are met in an individual if any of the following is present: 1. A pathogenic heterozygous NF1 variant with a variant allele fraction of significantly <50% in apparently normal tissue such as white blood cells AND one other NF1 diagnostic criterion (except a parent fulfilling diagnostic criteria for NF1) 2. An identical pathogenic heterozygous NF1 variant in two anatomically independent affected tissues (in the absence of a pathogenic NF1 variant in unaffected tissue) 3. A clearly segmental distribution of café-au-lait macules or cutaneous neurofibromas AND a. Another NF1 diagnostic criterion (except a parent fulfilling diagnostic criteria for NF1) or b. Child fulfilling diagnostic criteria for NF1 4. Only one NF1 diagnostic criterion from the following list: freckling in the axillary and inguinal region, optic pathway glioma, two or more Lisch nodules or two or more choroidal abnormalities, distinctive osseous lesions typical for NF1, two or more neurofibromas, or one plexiform neurofibroma AND a child fulfilling the criteria for NF1
Mosaic Legius syndrome	The diagnostic criteria for mosaic Legius syndrome are met in an individual if any of the following is present: 1. A heterozygous pathogenic SPRED1 variant with a variant allele fraction of significantly <50% in apparently normal tissue such as white blood cells AND six or more café-au-lait macules 2. An identical pathogenic heterozygous SPRED1 variant in two independent affected tissues (in the absence of a pathogenic SPRED1 variant in unaffected tissue) 3. A clearly segmental distribution of café-au-lait macules AND a child fulfilling the criteria for Legius syndrome

Contd...

Table 4: Contd...

RASopathy	Major criteria
Noonan syndrome (scoring system) (Definitive diagnosis: 1 "A" plus one other major sign or two minor signs; 1 "B" plus two major signs or three minor signs)	Major criteria (A) • Typical facial dysmorphism • Pulmonary stenosis, hypertrophic cardiomyopathy, and/or ECG finding typical of Noonan syndrome • Height <third percentile • Pectus carinatum/excavatum • First-degree relative with definite NS • Mental retardation, cryptorchidism and lymphatic dysplasia Minor criteria (B) • Suggestive facial dysmorphism • Other cardiac defects • Height <10 percentile • Broad thorax • First-degree relative with suggestive NS • One of mental retardation, cryptorchidism, and lymphatic dysplasia
Noonan syndrome with multiple lentigines/LEOPARD syndrome (Lentigines, electrographically conduction defects, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth, deafness)	(minimum criteria) If the patient has lentigines: features in at least two of the following categories are required for clinical diagnosis • Structural cardiac anomalies • Electrocardiographic cardiac involvement • Cardiac symptoms • Skeletal abnormality • Short stature • Genitourinary tract abnormality • Endocrine defects • Neurological defects • Cephalofacial dysmorphism If the patient has no lentigines: features in three of the above categories+an immediate relative with Noonan syndrome with multiple lentigines

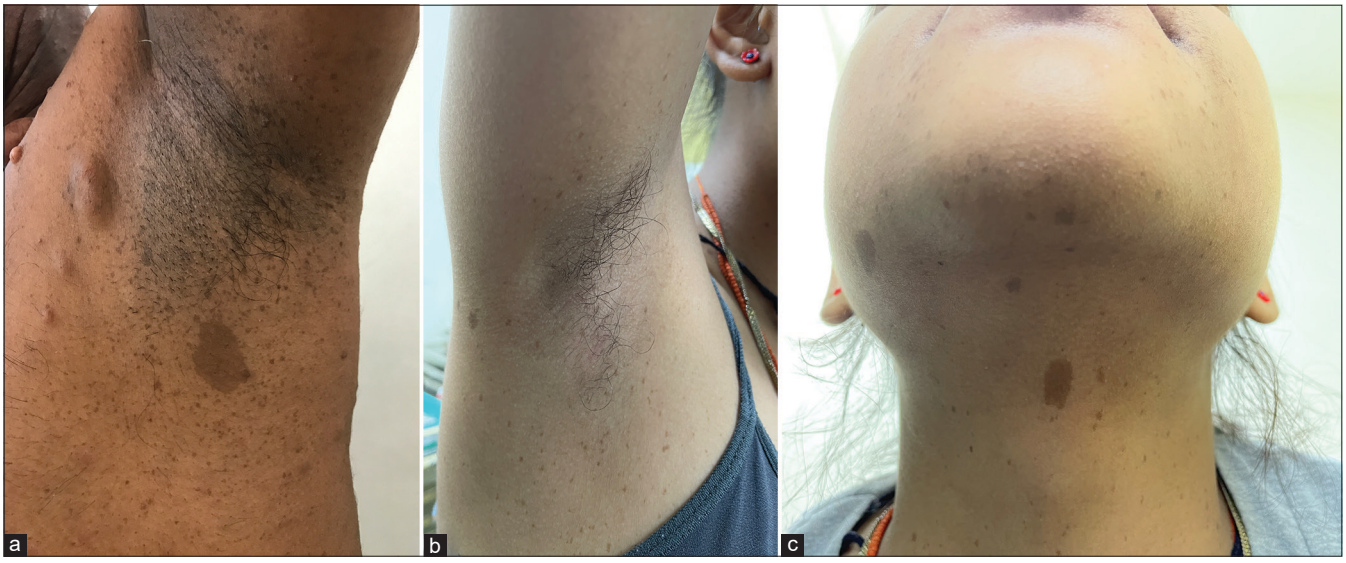

Figure 2: (a) Café-au-lait macules, axillary freckling and neurofibroma in an adult patient who fulfilled the diagnostic criteria for neurofibromatosis 1. (b and c) a possible case of Legius syndrome with multiple café-au-lait macules and axillary freckling, but absence of neurofibroma; her father also had similar lesions with no neurofibroma

intelligence, cardiovascular anomalies, peripheral nerve sheath tumors-16–26%.

Patients of NF 1 could have vasculopathy as well as renovascular hypertension because of renal artery stenosis. Patients may suffer from neurocognitive abnormalities, epilepsy, and intracranial neural tumors. Lisch nodules are characteristic features and are seen as 1–2 mm dome-shaped yellowish-brown nodules on the iris. In the oral cavity, one may develop neurofibroma and gingival overgrowth. Endocrinological abnormalities like precocious puberty, gynecomastia, acromegaly, Addison's disease, and hyperparathyroidism can occur in patients with NF 1.

Subtle indicators of malignant change in existing lesions include an increase in size, pain, and newly developed neuro-deficit.[32,33]

Noonan syndrome-Neurofibromatosis type 1 (NS NF 1)

This syndrome is considered to be *forme fruste* of NF 1[2]. Patients usually develop overlapping features of both the syndromes-cutaneous features of NF 1 with NS-like symptoms of growth retardation, congenital heart defects (CHD), and pectus excavatum/carinatum.

Legius syndrome (LS)

Apart from the classical features like CALM and axillary freckling [Figure 2b and c], the patient may have mild facial dysmorphism and learning disability. The patient could also have NS-type facial features and pectus excavatum.[1,34] Cardiac anomalies are rarely seen. Though there are no increased chances of malignancy, few reports of optic glioma are there.[35]

Costello syndrome (CS)

Patients with CS usually present with feeding difficulties and failure to thrive.[1,36] Because of overlapping features with NS and CFCS, there is always diagnostic difficulty at initial presentation, as the characteristic cutaneous features appear late. Distinctive facial features include coarse facies, hirsute forehead and eyebrows, macrocephaly, and epicanthal folds. These findings accentuate with time, and as the child ages, they will have large mouth with thick lips, prominent forehead, chubby cheeks, depressed nasal bridge, and posteriorly rotated low set ears. The child suffers from moderate cognitive and facial expression deficits.

Cardio-facio-cutaneous syndrome (CFCS)

Like CS, patients of CFCS also present with dysmorphic facial features, failure to thrive, and feeding difficulties. However, the presence of severe mental retardation and ectodermal anomalies are suggestive of CFCS.[1,37] Scalp eczema with absent scalp hair is another characteristic feature that might help to suspect this syndrome.[38] Unlike CS, patients with CFCS commonly have congenital heart defects (84%) than hypertrophic cardiomyopathy. Ocular abnormalities like strabismus, myopia, nystagmus, hyperopia, and astigmatism are common. The patient suffers from varying degrees of hypotonia, motor delay, seizures, speech delay, and learning disability. Patients with mutations in *KRAS* have a lower frequency of skin manifestations,[39] while cases having a mutation in MEK have normal cognitive development.[40]

Making a probable differential diagnosis based on cutaneous features is illustrated in Figure 3.

Capillary malformation-arteriovenous malformation syndrome (CM-AVM syndrome)

It is characterized by multiple CM with AVM involving various organs—skin, muscle [Figure 4], bone, heart, and brain.[41,42] Intracranial and spinal AVM are associated with poor prognosis.[43] Mutations in *RASA 1* have also been reported in Parkes Weber syndrome and vein of Galen malformations. Hence, these are close differential diagnoses of CM-AVM syndrome.

Mosaic RASopathies

RAS/MAPK pathway mutations have been found to involve non-melanocytic and melanocytic nevi as well as nevus-related syndromes.[2] Nevoid cutaneous lesions are a classical example of mosaicism. The term "Mosaic RASopathies" was first used by Hafner who expanded the concept of RASopathies beyond germline mutations.[8] Sebaceous nevi [SN-Figure 5a] can be seen in the mosaic pattern. Whenever SN is associated with extracutaneous features like cerebral, ocular, and skeletal defects, it is known as Schimmelpenning syndrome (SS). The risk of malignancy in SN is 8–23%. Groesser *et al.*[44] has reported that 95% of SN demonstrated *HRAS* mutations. Maybe this explains the higher chances of malignancy in this nevus.

Various studies have reported that a third to 39% of patients with epidermal keratinocytic nevus [EKN-Figure 5b] harbor mutations in the concerned pathway. Few cases of EKN with malignancy involving other organs like urothelial carcinoma and rhabdomyosarcoma have been reported. These patients had mutations of *RAS* genes in EKN. Though the malignant potential of mosaic mutations has been not studied well, extensive cutaneous involvement with extracutaneous features should prompt vigilance in the future. Also, the timing of mutation—before or after the development of the epidermis—is important in regards that the patient will develop phacomatosis pigmentovascularis or phacomatosis pigmentokeratotica, respectively.[44]

Mosaic NF 1 [Figure 5c] and LS have been described, and diagnostic criteria are discussed in Table 4. Various melanocytic nevi—agminated segmental nevi, nevus spilus, nevus spilus type congenital melanocytic nevi [Figure 5d], and nevus spilus with agminated spitz nevi—have shown mutations in *RAS* and *RAF* genes.[45,46] Development of

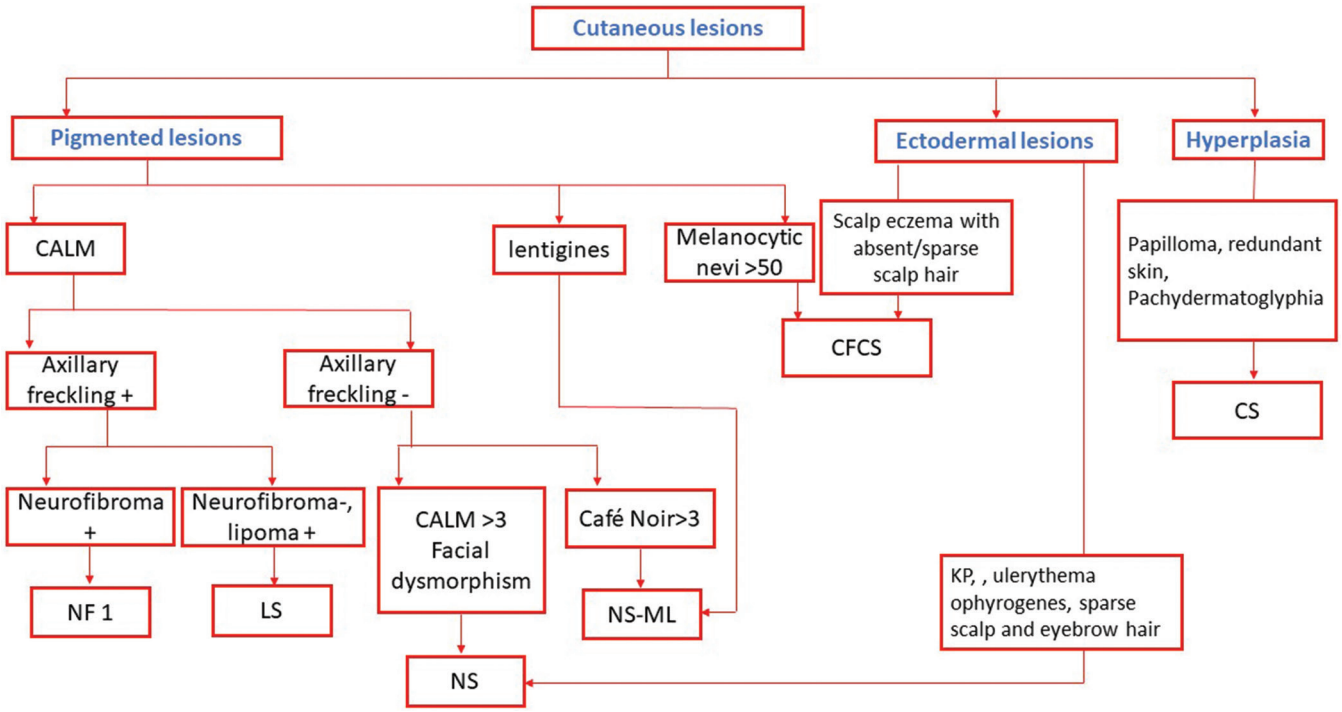

Figure 3: Diagnostic flow chart based on cutaneous features. NF 1- Neurofibromatosis 1, LS- Legius syndrome, NS- Noonan syndrome, NS-ML- Noonan syndrome with multiple lentigines, CFCS- Cardiofaciocutaneous syndrome, CS- Costello syndrome

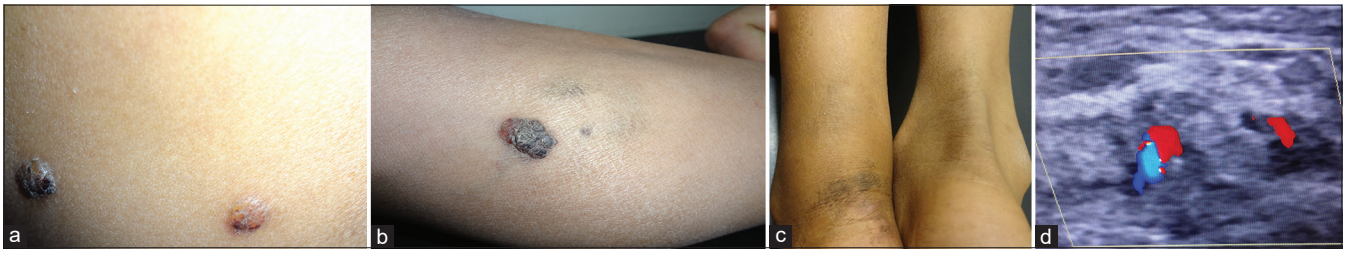

Figure 4: A possible case of capillary malformation- arteriovenous malformation syndrome (a) Capillary malformation (b) Capillary malformation with swelling in calf (c) Swelling in ankle region due to arteriovenous malformation (d) Doppler study showing arteriovenous malformation in calf muscles

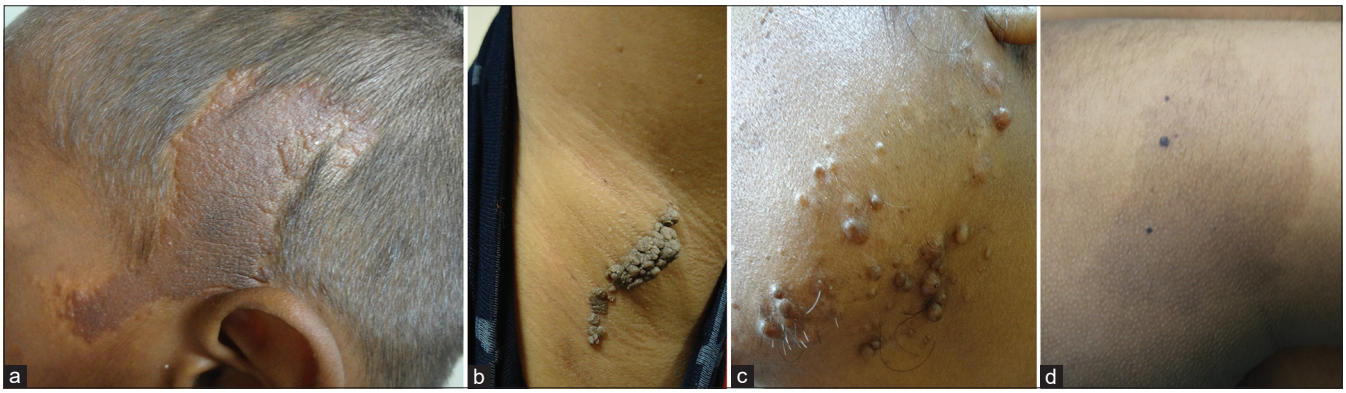

Figure 5: (a) Sebaceous nevi (b) Epidermal keratinocytic nevi (c) Segmental neurofibromatosis 1 (d) Nevus spilus-type congenital melanocytic nevi

secondary tumors or secondary lesions[47] has shown copy number gain.

CCLA (central conducting lymphatic anomalies syndrome)

This is a recently described entity that is characterized by lower limb edema which may be eventually followed by lymph accumulation in the genital area, thorax, pericardium, and abdomen. Dilatation of central lymphatic channels and or leakage of the same may result in the above features. The most common etiology behind this is germline and mosaic RASopathies.

Management

Management of RASopathies will be briefly discussed under headings of prenatal diagnosis, genetic diagnosis, other investigations, cancer surveillance, and possible treatment options.

Prenatal diagnosis

A recent study done by Scott *et al.*[48] used parallel or Sanger sequencing for identifying various mutated genes in prenatal diagnosis. They suggested that a large molecular panel should be offered to test for RASopathies' gene mutations as one might miss a particular gene mutation if it is not included in the panel. Also, correlation between prenatal and postnatal phenotype should be performed. Both the two major studies[48,49] recommended testing on the fetus whenever the following findings are seen on prenatal ultrasonography (to be done in both 1st and 2nd trimesters to see the persistence of a few indicators).

- Significantly higher nuchal translucency (NT > 6 mm)
- Increased nuchal fold (>6 mm)
- Cystic hygroma
- Thoracic effusion
- Hydrops fetalis
- Congenital heart defects
- Lymphatic anomalies
- If the NT is increased but <6, and other suggestive features like—polyhydramnios, renal anomalies, and ascites.

Genetic testing

Genetic analysis with next-generation sequencing (NGS) can be done from blood or from tissue samples (especially in the case of mosaic RASopathies), which can further be confirmed with Sanger sequencing. Leung GKC *et al.*[50] suggested that functional genetic diagnostic analysis can be integrated along with NGS to further improve diagnostic yield as well as genotyping and phenotyping correlation.

Other investigations

Patients with RASopathies need to be evaluated for various other systemic associations. A coagulation profile can be obtained in cases of NS.[7] For various other systemic involvement and organ specific malignancies imaging techniques—X-ray, computed tomography, magnetic resonance, echocardiography, electrocardiography—can be done at baseline and repeated whenever necessary. For large vascular malformations color Doppler studies can be obtained. Also, patients need to be evaluated for features of autoimmunity and endocrinological disorders if clinical symptoms are suggestive.

Cancer surveillance

Patients with NS and NF 1 should be evaluated for hematological malignancy; hence, complete blood count and peripheral smear can be done on presentation and repeated whenever necessary.[7] The splenic and hepatic examination is recommended in cases of NS. Urine analysis, nasal endoscopy, ear examination, chest X-ray cystoscopy, and ultrasonography are recommended in CS. The overall frequency of surveillance should be every 3–6 months for every high-risk mutation.[51] No routine surveillance is recommended in patients of CFCS, NS-ML, NS-LAH, and LS.

Possible treatment options

Treatment of RASopathies is presently mainly under clinical development with the exception of a few drugs.[5] However, preclinical trials (on animal and cell models—Zebrafish, *Drosophila*, *Caenorhabditis elegans*, knock-in mouse models, induced pluripotent stem cells) for many drugs are undergoing. Hopefully, in the near future, we will have medicines available to treat these syndromes. Drugs can be broadly classified into the following categories.

A. Inhibitors of *RAS* activation:
   - Inhibitors of prenylation in turn preventing membrane localization of RAS: Alendronate, Pamidronate, Lovastatin, Atorvastatin, and Tipifarnib.
   - Inhibitors of RAS and SOS1 interactions: RMC-4630 (yet not tested on models).

B. Direct *RAS* inhibitors: Adagrasib, Sotoracib, ASO/SSO, BI 2852.

C. RAS/MAPK pathway inhibitors:
   - RAF inhibitors: Belvarafenib, LY3009120, LXH254.
   - MEK inhibitors: Binimetinib, Cobimetinib, Mirdametinib, Selumetinib, Trametinib.
   - ERK inhibitors: Ulixertinib.

Selumetinib is the only drug that is FDA-approved in patients of RASopathies for its use in plexiform neurofibroma.[52] It has been shown to improve quality of life and pain as well as reduce the size of plexiform neurofibroma especially when surgical therapies are not feasible in paediatric patients.[52,53] Commonest side effects reported with its use are rash, acneiform eruptions, headache, nausea, vomiting, paronychia, and elevated levels of creatinine phosphokinase levels. Other drugs like sotoracib and trametinib are approved for their use in cancer, and statins as well as N-bisphosphonates are FDA approved for other diseases. So, off-label use of these drugs in RASopathies has been done by few clinicians with improvement in various features. Trametinib have been tried in two infants who had *RIT 1* associated NS, and they showed significant improvement.[54] Apart from above mentioned drugs many specific inhibitors are under development and have shown promising results in preclinical trials. Also, a few novel therapies like copper chelators[55] and tyrosine kinase inhibitors like dasatinib,[56] etc., have been reported to be beneficial in preclinical trials. Inhibitors of the mTOR pathway like everolimus have been tried in the treatment of CM-AVM syndrome

in animal models and in patients of NSML,[57] and PIK3 inhibitors (alpelibsib, etc.) or AKT inhibitors (miransertib, etc.) have been used in other RASopathies.

Though most of these RASopathies are rare, a few of them like NF 1 are not so rare. NGS has identified RAS/MAPK pathway gene mutations in a few recently described entities and so the spectrum of RASopathies is broadening. Being dermatologists, we can contribute to the diagnosis of these complex syndromes which requires a multidisciplinary approach by many other specialties. Apart from genetic analysis, many other hematological and radiological investigations are required for monitoring the patients and to rule out malignancy. The availability of drugs in the future will be life-changing for many patients with RASopathies who have multisystem involvement and associated morbidity. Genetic counseling of family and prenatal diagnosis are also important parts of the management of these morbid conditions.

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Conflicts of interest

There are no conflicts of interest.

References

1. Hernández-Martín A, Torrelo A. [Rasopathies: Developmental disorders that predispose to cancer and skin manifestations]. Actas Dermo-sifiliograficas 2011;102:402-16.
2. Jafry M, Sidbury R. RASopathies. Clin Dermatol 2020;38:455-61.
3. Riller Q, Rieux-Laucat F. RASopathies: From germline mutations to somatic and multigenic diseases. Biomed J 2021;44:422-32.
4. Prior IA, Lewis PD, Mattos C. A comprehensive survey of Ras mutations in cancer. Cancer Res 2012;72:2457-67.
5. Hebron KE, Hernandez ER, Yohe ME. The RASopathies: From pathogenetics to therapeutics. Dis Model Mech 2022;15:dmm049107.
6. Rauen KA. Defining RASopathy. Dis Model Mech 2022;15:dmm049344.
7. Palit A, Inamadar AC. RASopathies: Dermatologists' viewpoints. Indian J Dermatol Venereol Leprol 2022;88:452-63.
8. Luo S, Tsao H. Epidermal, sebaceous, and melanocytic nevoid proliferations are spectrums of mosaic RASopathies. J Invest Dermatol 2014;134:2493-6.
9. Engler M, Fidan M, Nandi S, Cirstea IC. Senescence in RASopathies, a possible novel contributor to a complex pathophenoype. Mech Ageing Dev 2021;194:111411.
10. Castillo-Quan JI, Tain LS, Kinghorn KJ, Li L, Grönke S, Hinze Y, *et al*. A triple drug combination targeting components of the nutrient-sensing network maximizes longevity. Proc Natl Acad Sci U S A 2019;116:20817-9.
11. van Deursen JM. Senolytic therapies for healthy longevity. Science 2019;364:636-7.
12. Gripp KW, Lin AE. Costello syndrome: A Ras/mitogen activated protein kinase pathway syndrome (rasopathy) resulting from HRAS germline mutations. Genet Med 2012;14:285-92.
13. White SM, Graham JM Jr, Kerr B, Gripp K, Weksberg R, Cytrynbaum C, *et al*. The adult phenotype in Costello syndrome. Am J Med Genet A 2005;136:128-35.
14. Valencic E, Da Lozzo P, Tornese G, Ghirigato E, Facca F, Piscianz E, *et al*. Clinical and cytometric study of immune involvement in a heterogeneous cohort of subjects with RASopathies and mTORopathies. Front Pediatr 2021;9:703613.
15. Redmond MT, Scherzer R, Prince BT. Novel genetic discoveries in primary immunodeficiency disorders. Clin Rev Allergy Immunol 2022;63:55-74.
16. Siano MA, Marchetti V, Pagano S, Di Candia F, Alessio M, De Bsrsi D, *et al*. Risk of autoimmune diseases in patients with RASopathies: Systematic study of humoral and cellular immunity. Orphanet J Rare Dis 2021;16:410.
17. Pevec U, Rozman N, Gorsek B, Kunej T. RASopathies: Presentation at the genome, interactome, and phenome levels. Mol Syndromol 2016;7:72-9.
18. Rauen KA. The RASopathies. Annu Rev Genomics Hum Genet 2013;14:355-69.
19. Zenker M. Clinical overview on RASopathies. Am J Med Genet C Semin Med Genet 2022;190:414-24.
20. Martínez-Quintana E, Rodríguez-González F. LEOPARD syndrome: Clinical features and gene mutations. Mol Syndromol 2012;3:145-57.
21. Legius E, Messiaen L, Wolkenstein P, Pancza P, Avery RA, Berman Y, *et al*. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: An international consensus recommendation. Genet Med 2021;23:1506-13.
22. Voron DA, Hatfield HH, Kalkhoff RK. Multiple lentigines syndrome. Case report and review of the literature. Am J Med 1976;60:447-56.
23. van der Burgt I. Noonan syndrome. Orphanet J Rare Dis 2007;2:4.
24. Nugent DJ, Romano AA, Sabharwal S, Cooper DL. Evaluation of bleeding disorders in patients with Noonan syndrome: A systematic review. J Blood Med 2018;9:185-92.
25. Digilio MC, Conti E, Sarkozy A, Mingarelli R, Dottorini T, Marino B, *et al*. Grouping of multiple-lentigines/LEOPARD and Noonan syndromes on the PTPN11 gene. Am J Hum Genet 2002;71:389-94.
26. van Trier D, van der Burgt I, Draaijer RW, Cruysberg JR, Noordam C, Draaisma JM. Ocular findings in Noonan syndrome: A retrospective cohort study of 105 patients. Eur J Pediatr 2018;177:1293-8.
27. Kim YE, Baek ST. Neurodevelopmental aspects of RASopathies. Mol Cells 2019;42:441-7.
28. Rodríguez-Bujaldón A, Vazquez-Bayo C, Jimenez-Puya R, Galen-Gutierrez M, Moreno-Giemenez J, Rodriguez-Garcia A, *et al*. LEOPARD syndrome: What are Café Noir spots? Pediatr Dermatol 2008;25:444-8.
29. Digilio MC, Lepri F, Baban A, Dentici ML, Versacci P, Capolino R, *et al*. Rasopathies: Clinical diagnosis in the first year of life. Mol Syndromol 2010;1:282-9.
30. Carcavilla A. The value of dermatological phenotyping in the clinical diagnosis of RASopathies. Br J Dermatol 2019;180:1284-301.
31. Tafazoli A, Eshraghi P, Koleti ZK, Abbaszadegan M. Noonan syndrome-a new survey. Arch Med Sci 2017;13:215-22.
32. Brems H, Beert E, deRavel T, Legius E. Mechanisms in the pathogenesis of malignant tumours in neurofibromatosis Type 1. Lancet Oncol 2009;10:508-15.
33. Bergqvist C, Servy A, Valeyrie-Allanore L, Ferkel S, Combemale P, Wolkenstein P, *et al*. Neurofibromatosis 1. French

national guidelines based on an extensive literature review since 1966. Orphanet J Rare Dis 2020;15:37.
34. Cao H, Alrejaye N, Klein OD, Goodwin AF, Oberoi S. A review of craniofacial and dental findings of the RASopathies. Orthod Craniofac Res 2017;20:32-8.
35. Skrypek M, Bendel A, Kebriaei M, Mendelsohn N, Wein A, Hansen M, *et al*. Case report of an optic pathway glioma in a patient with Legius syndrome. Neurooncology 2016;18:iii78-96.
36. Rauen KA, Banerjee A, Bishop WR, Lauchle JO, Mccormick F, McMahon M, *et al*. Costello and Cardio-Facio-Cutaneous syndromes: Moving toward clinical trials in RASopathies. Am J Med Genet C Semin Med Genet 2011;157C: 136-46.
37. Pierpont ME, Magoulas PL, Adi S, Kavamura MI, Neri G, Noonan J, *et al*. Cardio-facio-cutaneous syndrome: Clinical features, diagnosis, and management guidelines. Pediatrics 2014;134:e1149-62.
38. Rocha VB, Moraes RA, Pereira LB. Cardiofaciocutaneous syndrome and the dermatologist's contribution to diagnosis. Cutis. 2017;99:E4-E7
39. Niihori T, Aoki Y, Narumi Y, Neri G, Cavé H, Verloes A, *et al*. Germline KRAS and BRAF mutations in cardio-facio-cutaneous syndrome. Nat Genet 2006;38:294-6.
40. Nava C, Hanna N, Michot C, Pereira S, Pouvreau N, Niihori T, *et al*. Cardio-facio-cutaneous and Noonan syndromes due to mutations in the RAS/MAPK signalling pathway: Genotype-phenotype relationships and overlap with Costello syndrome. J Med Genet 2007;44:763-71.
41. Boon LM, Mulliken JB, Vikkula M. RASA1: Variable phenotype with capillary and arteriovenous malformations. Curr Opin Genet Dev 2005;15:265-9.
42. Eerola I, Boon LM, Mulliken JB, Burrows PE, Dompmartin A, Watanabe S, *et al*. Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. Am J Hum Genet 2003;73:1240-9.
43. Cai R, Liu F, Hua C, Yu Z, Ramien M, Malic C, *et al*. A novel RASA1 mutation causing capillary malformation-arteriovenous malformation (CM-AVM): The first genetic clinical report in East Asia. Hereditas 2018;155:24.
44. Groesser L, Herschberger E, Sagrera A, Shwayder T, Flux K, Ehmann L, *et al*. Phacomatosis pigmentokeratotica is caused by a postzygotic HRAS mutation in a multipotent progenitor cell. J Invest Dermatol 2013;133:1998-2003.
45. Kinsler VA, Krengel S, Riviere JB, Waelchli R, Chapusot C, Al-Olabi L, *et al*. Next-generation sequencing of nevus spilus-type congenital melanocytic nevus: Exquisite genotype-phenotype correlation in mosaic RASopathies. J Invest Dermatol 2014;134:2658-60.
46. Sarin KY, McNiff JM, Kwok S, Kim J, Khavari PA. Activating HRAS mutation in nevus spilus. J Invest Dermatol 2014;134:1766-8.
47. Sarin KY, Sun BK, Bangs CD, Cherry A, Swetter SM, Kim J, *et al*. Activating HRAS mutation in agminated Spitz nevi arising in a nevus spilus. JAMA Dermatol 2013;149:1077-81.
48. Scott A, Di Giosaffatte N, Pinna V, Daniele P, Corno S, D'Ambrosio V, *et al*. When to test fetuses for RASopathies? Proposition from a systematic analysis of 352 multicenter cases and a postnatal cohort. Genet Med 2021;23:1116-24.
49. Stuurman KE, Joosten M, van der Burgt I, Elting M, Yntema HG, Meijers-Heijboer H, *et al*. Prenatal ultrasound findings of RASopathies in a cohort of 424 fetuses: Update on genetic testing in the NGS era. J Med Genet 2019;56:654-61.
50. Leung GKC, Luk HM, Tang VHM, Gao WW, Mak CCY, Yu MHC, *et al*. Integrating functional analysis in the next-generation sequencing diagnostic pipeline of RASopathies. Sci Rep 2018;8:2421.
51. Ney G, Gross A, Livinski A, Kratz CP, Stewart DR. Cancer incidence and surveillance strategies in individuals with RASopathies. Am J Med Genet C Semin Med Genet 2022;190:530-40.
52. Gross AM, Wolters PL, Dombi E, Baldwin A, Whitcomb P, Fisher MJ, *et al*. Selumetinib in children with inoperable plexiform neurofibromas. N Engl J Med 2020;382:1430-42.
53. Gross AM, Glassberg B, Wolters PL, Dombi E, Baldwin A, Fisher MJ, *et al*. Selumetinib in children with neurofibromatosis type 1 and asymptomatic inoperable plexiform neurofibroma at risk for developing tumor-related morbidity. Neuro Oncol 2022;24:1978-88.
54. Andelfinger G, Marquis C, Raboisson MJ, Théoret Y, Waldmüller S, Wiegand G, *et al*. Hypertrophic cardiomyopathy in Noonan syndrome treated by MEK-inhibition. J Am Coll Cardiol 2019;73:2237-9.
55. Brady DC, Crowe MS, Greenberg DN, Counter CM. Copper chelation inhibits BRAFV600E-driven melanomagenesis and counters resistance to BRAFV600E and MEK1/2 inhibitors. Cancer Res 2017;77:6240-52.
56. Yi JS, Huang Y, Kwaczala AT, Kuo IY, Ehrlich BE, Campbell SG, *et al*. Low-dose dasatinib rescues cardiac function in Noonan syndrome. JCI Insight 2016;1:e90220.
57. Hahn A, Lauriol J, Thul J, Behnke-Hall K, Logeswaran T, Schänzer A, *et al*. Rapidly progressive hypertrophic cardiomyopathy in an infant with Noonan syndrome with multiple lentigines: Palliative treatment with a rapamycin analog. Am J Med Genet A 2015;167A:744-51.