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REVIEW

# Recent advances in RASopathies

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**RASopathies or RAS/mitogen-activated protein kinase (MAPK) syndromes are a group of phenotypically overlapping syndromes caused by germline mutations that encode components of the RAS/MAPK signaling pathway. These disorders include neurofibromatosis type I, Legius syndrome, Noonan syndrome, Noonan syndrome with multiple lentigines (formerly called LEOPARD syndrome), Costello syndrome, cardiofaciocutaneous (CFC) syndrome, Noonan-like syndrome, hereditary gingival fibromatosis and capillary malformation–arteriovenous malformation. Recently, novel gene variants, including *RIT1*, *RRAS*, *RASA2*, *A2ML1*, *SOS2* and *LZTR1*, have been shown to be associated with RASopathies, further expanding the disease entity. Although further analysis will be needed, these findings will help to better elucidate an understanding of the pathogenesis of these disorders and will aid in the development of potential therapeutic approaches. In this review, we summarize the novel genes that have been reported to be associated with RASopathies and highlight the cardiovascular abnormalities that may arise in affected individuals.**
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## INTRODUCTION

RAS is a member of small GTPases that regulate cell growth, proliferation and differentiation. RAS GTPases convey an extracellular signal to its target of effector proteins in cells. RAS cycles between the guanosine diphosphate (GDP)-bound inactive form and the guanosine triphosphate (GTP)-bound active form. GTP-bound RAS utilizes several downstream effectors, including RAF1, PI-3 kinase, PLCε and Ral-GDS.[1]

The RAS/mitogen-activated protein kinase (MAPK) pathway is an essential signaling pathway that controls cell proliferation, differentiation and survival. Numerous studies have revealed that dysregulation of the RAS/MAPK pathway causes clinically overlapping genetic disorders, termed 'RASopathies' or 'RAS/MAPK syndromes'.[2,3] Although each RASopathy has a unique phenotype, these syndromes have many overlapping characteristics, including craniofacial dysmorphology, cardiovascular abnormalities, musculoskeletal abnormalities, cutaneous lesions, neurocognitive impairment and increased risk of tumor (for a review of the details of each of these disorders, see Rauen[4]). These disorders include the following: (1) neurofibromatosis type 1 (NF1) caused by haploinsufficiency of neurofibromin;[5–7] (2) NF1-like syndrome caused by haploinsufficiency of *SPRED1*;[8] (3) Noonan syndrome (NS) caused by mutations in *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *BRAF* and *NRAS*;[9–15] (4) NS with multiple lentigines (NSML) caused by mutations in *PTPN11* and *RAF1*;[9,16] (5) Costello syndrome caused by activating mutations in *HRAS*;[17] (6) cardiofaciocutaneous (CFC) syndrome caused by mutations in *BRAF*, *MAP2K1/2* and *KRAS*;[18,19] (7) Noonan-like syndrome caused by mutations in *SHOC2*[20] or *CBL*;[21–23] (8) hereditary gingival fibromatosis caused by a mutation in *SOS1*;[24] and (9) capillary malformation–arteriovenous malformation caused by haploinsufficiency of *RASA1* (also known as p120 Ras-GTPase activating protein (GAP)).[25] Molecular analysis is beneficial for both the confirmation of clinical diagnoses and to perform follow-up according to the unique characteristics of each disorder. In this review, we summarize novel genes that have been reported to be associated with RASopathies, including *RIT1*, *RRAS*, *RASA2*, *A2ML1*, *SOS2* and *LZTR1*, and discuss the cardiovascular abnormalities that have been associated with these syndromes.

## NOVEL GENES ASSOCIATED WITH RASOPATHIES

NS (MIM 163950) is an autosomal dominant disorder that is characterized by short stature, facial dysmorphism and congenital heart defects.[26] The incidence of this syndrome is estimated to be between 1 in 1000 and 1 in 2500 live births.[27] The distinctive craniofacial features that are observed in individuals with NS include a webbed or short neck, hypertelorism, downslanting palpebral fissures, ptosis and low-set, posteriorly rotated ears (see reviews[26,28]). More than 80% of individuals with NS have cardiovascular involvement, most frequently including congenital heart diseases, pulmonary valve stenosis and hypertrophic cardiomyopathy.[26] Hypertrophic cardiomyopathy is observed in ~20% of individuals.[26,28] Other clinical manifestations include cryptorchidism, bleeding disorders, mild neurocognitive delay and pectus deformity. NS is known to be associated with myeloproliferative disorders. The myeloproliferative disorders most often resolve spontaneously, although select individuals develop juvenile myelomonocytic leukemia, a myeloproliferative disorder characterized by excessive production of myelomonocytic cells.[26,28] As of 2013, seven genes have been shown to be associated with NS: *PTPN11* (~50%), *SOS1* (11%), *RAF1* (5%), *KRAS* (~1.5%), *NRAS* (0.2%), *SHOC2*

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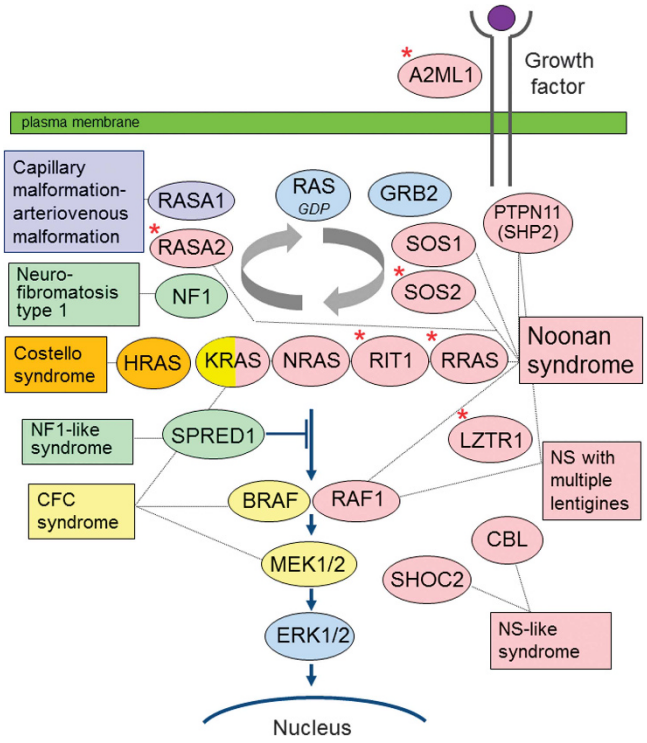


**Figure 1** RAS/MAPK cascade and disorders involving germline mutations of related genes. MAPK, mitogen-activated protein kinase; NF1, neurofibromatosis type 1; NS, Noonan syndrome. *Indicates possible causative genes that have been reported since 2013.

(~2%) and *CBL* (Figure 1).[26] However, it is estimated that 20–30% of the causative genes behind NS and NS-like disorders are unidentified. Recent advances in genetic analysis technologies, including whole-exome sequencing, have identified potential causes for RASopathies.

### RIT1

Our group performed whole-exome sequencing of 14 individuals with NS and related conditions who had no detectable mutations in known Noonan-related genes. We found four variants in *RIT1* that were clustered within 14 amino acids. Combining these data with additional Sanger sequencing data revealed a total of nine missense, nonsynonymous *RIT1* mutations in 17 of a group of 180 individuals (9%) (Table 1).[29] The RIT1 protein shares ~50% sequence identity with RAS; comparatively, it has an additional N-terminal extension and does not possess a carboxyl-terminal CAAX motif.[30,31] Past studies have shown that a RIT1 p.Q79L mutant that corresponds to RAS p.Q61L is implicated in transforming NIH3T3 cells, modulating neurite outgrowth in neuronal cells, and activating extracellular-signal-regulated kinase (ERK) and p38 MAPK in a cell-specific manner.[32–34] The mutations in *RIT1* that have been identified in individuals with NS are located in its G1 domain (p.S35T) and in the switch I region that is included in its G2 domain (p.A57G). The majority of the mutations (p.E81G, p.F82V, p.F82L, p.T83P, p.Y89H, p.M90I and p.G95A) are clustered within the switch II region that corresponds to RAS. Seventy-percent of mutation-positive individuals had hypertrophic cardiomyopathy, representing a high frequency of individuals with NS. The introduction of mutant *RIT1* mRNAs into one-cell stage zebrafish embryos was demonstrated to result in a significant increase of embryos with craniofacial abnormalities, incomplete looping and hypoplastic chambers in the heart, and elongated yolk sacs.[29]

Following the initial report, Chen *et al.*[35] performed whole-exome sequencing of 27 individuals with NS who did not possess mutations in the genes known to be associated with NS. They identified missense mutations in *RIT1* (p.A57G, p.A77P, p.F82V and p.G95A) in five individuals with NS. Bertola *et al.*[36] and Gos *et al.*[37] identified *RIT1* mutations in 6 out of 70 individuals and 4 out of 106 individuals, respectively. In total, 10 different *RIT1* mutations have been reported in 32 individuals. The most frequent mutation in *RIT1* is p.G95A (10 out of 32 individuals). Out of 32 *RIT1* mutation-positive individuals, 16 (50%) showed cardiac hypertrophy. Both these results and unpublished data produced by our group suggest that the frequency of *RIT1* mutations can be estimated as ~5% in patients with NS, similar to the frequency of *RAF1* mutations in these patients. Although somatic *RIT1* mutations have previously been considered to be rare in cancer patients, recent reports have identified somatic *RIT1* mutations in ~2% of lung adenocarcinomas[38,39] and myeloproliferative or mixed myelodysplastic/myeloproliferative neoplasms, particularly in chronic myelomonocytic leukemia.[40]

### RRAS

Flex *et al.*[41] identified two germline mutations (p.G39dup and p.V55M) in *RRAS*, a member of the RAS subfamily,[42] in two individuals with NS. Germline mutations in *RRAS* are rare (2 subjects out of 504 individuals with NS and related disorders). They also identified somatic *RRAS* mutations in 2 out of 110 samples taken from patients with juvenile myelomonocytic leukemia. The expression of the identified *RRAS* mutations in *Caenorhabditis elegans* resulted in enhanced RAS signaling and phenotypic abnormalities, similar to what is observed in *C. elegans* that are expressing a NS causing *SHOC2* mutant.[20]

### RASA2

Chen *et al.*[35] identified *RASA2* variants in three individuals with NS. RASA2 is a member of the mammalian RAS-GAP family. Loss-of-function mutations in *NF1* and *RASA1*, which are also RAS-GAPs, have been identified in individuals with NF1 and capillary malformation–arteriovenous malformation, respectively.[6,7,25] All of the identified variations in *RASA2* (p.Y326C, p.Y326N and p.R511C) affect highly conserved amino acids in the GAP domain of RASA2. The expression of these mutants in HEK293T cells did not suppress ERK after EGF treatment, unlike in cells with wild-type RASA2. It was concluded that two variants were loss-of-function mutations and one variant was a dominant negative mutation. In contrast with *RASA1* (p120GAP), the functional role of *RASA2* has not yet been fully elucidated. According to the COSMIC database (http://cancer.sanger.ac.uk/cosmic), somatic missense and nonsense mutations in *RASA2* have been identified in various tumors, including those corresponding to colorectal, skin, lung and endometrial cancers.

### A2ML1

Vissers *et al.*[43] performed trio exome sequencing and identified a *de novo* variant (p.R802H) of *A2ML1* in an individual with NS. Additional analyses of 155 individuals revealed missense variants (p.R592L and p.R802L) of *A2ML1* in two families with NS. Introducing the identified mutations into zebrafish led to developmental defects, including a phenotype that exhibited a broad head, blunted face and cardiac malformations. The *A2ML1* gene encodes the secreted protease inhibitor α-2-macroglobulin-like-1, a member of the

**Table 1 Novel genes that have been shown to be potentially associated with RASopathies**

| *Gene symbol* | *Protein function* | *Nucleotide change* | *Amino acid change* | *De novo/familial* | *Number of families reported* | *References* |
|---|---|---|---|---|---|---|
| *RIT1* | RAS GTPase | c.104G>C | p.S35T | *De novo*/familial | 3 | 29,36 |
| | | c.170C>G | p.A57G | *De novo* | 7 | 29,35,36 |
| | | c.229G>C | p.A77P | Parental samples not available | 1 | 35 |
| | | c.242A>G | p.D81G | Parental samples not available | 1 | 29 |
| | | c.244T>G | p.F82V | *De novo* | 3 | 29,35,37 |
| | | c.246T>G | p.F82L | *De novo* | 3 | 29,36 |
| | | c.247A>C | p.T83P | *De novo* | 1 | 29 |
| | | c.265T>C | p.Y89H | Parental samples not available | 1 | 29 |
| | | c.270G>T,c.270G>C | p.M90I | *De novo* | 2 | 29,37 |
| | | c.284G>C | p.G95A | *De novo* | 10 | 29, 35–37 |
| *RRAS* | RAS GTPase | c.163G>A | p.V55M | Parental samples not available | 1 | 42 |
| | | c.116_118dupGCG | p.G39dup | *De novo* | 1 | 42 |
| *RASA2* | GTPase activating protein | c.976T>A | p.Y326N | Parental samples not available | 1 | 35 |
| | | c.977A>G | p.Y326C | Parental samples not available | 1 | 35 |
| | | c.1531C>T | p.R511C | Parental samples not available | 1 | 35 |
| *A2ML1* | α-Macroglobulin superfamily of proteins | c.1775G>T | p.R592L | Segregating in a family | 1 | 43 |
| | | c.2405G>A | p.R802H | *De novo* | 1 | 43 |
| | | c.2405G>T | p.R802L | Segregating in a family | 1 | 43 |
| *SOS2* | Guanine nucleotide exchange factor | c.1127C>G | p.T376S | Segregating in a family | 2 | 49 |
| | | c.800T>A | M267K | *De novo* | 1 | 49 |
| *LZTR1* | BTB-kelch superfamily | c.356A>G | p.Y119C | *De novo* | 1 | 49 |
| | | c.740G>A | p.S247N | Segregating in a family | 1 | 49 |
| | | c.742G>A | p.G248R | Segregating in a family | 1 | 49 |
| | | c.850C>T | p.R284C | *De novo* | 1 | 49 |
| | | c.859C>T | p.H287Y | *De novo* | 1 | 49 |

α-macroglobulin superfamily of proteins. This family contains components of the complement system and protease inhibitors.[44] The A2ML1 protein is expressed in epidermal granular keratinocytes and is secreted into extracellular space, where it demonstrates inhibitory activities toward proteases *in vitro*, including chymotrypsin and papain.[44] Such activities suggest that it has a role in the defense mechanisms and maintenance of epidermal homeostasis. It is notable that A2ML1 autoantibodies have frequently been detected in individuals with paraneoplastic pemphigus, an autoimmune multiorgan syndrome that includes intractable stomatitis, polymorphous cutaneous lesions and lymphoproliferative tumors.[45,46] A2ML1 has been shown to bind to LPR1 (low-density lipoprotein receptor-related protein 1).[47] LPR1 has been shown to interact with *CBL*, a causative gene of RASopathy, and it is known to control the ubiquitination of platelet-derived growth factor receptor-β.[48] Both the functional properties of A2ML1 and the mechanisms by which A2ML1 regulates the RAS/ERK pathway are largely unknown. Further functional analysis will clarify the role of A2ML1 in developmental disorders.

### SOS2

Yamamoto *et al.*[49] performed whole-exome sequencing of 50 Brazilian probands who were negative for the gene mutations known to be associated with NS. They identified two missense variants in *SOS2* in three families with NS. *De novo* occurrence was confirmed in one of three families. *SOS2* is homologous to *SOS1*, the second most frequently mutated gene in individuals with NS. The identified variants, p.M267K and T376S, were located in the DH domain of SOS2, and this is where the *SOS1* mutations that were identified in NS patients were also clustered, suggesting that these mutations could be pathogenic.

### LZTR1

Yamamoto *et al.*[49] have also identified rare variants of *LZTR1*, leucine-zipper-like transcription regulator 1, in individuals with NS. They concluded that five variants are predicted to cause NS; three of the variants, p.R284C, p.H287Y and p.Y119C, were confirmed to be *de novo* events and two of the variants, p.G248R and p.S247N, were found to be segregated in the affected individuals of two families. *LZTR1* encodes a protein member of the BTB-kelch superfamily that has not been previously associated with the RAS/MAPK pathway. Somatic and germline mutations in *LZTR1* have been identified in patients with glioblastoma multiforme[50] and multiple schwannomas[51] respectively. *LZTR1* is located within the 3-Mb-long region that is most commonly deleted in patients with 22q11 deletion syndrome.[52] In two individuals, Chen *et al.*[35] identified *LZTR1* p.R237Q and p.A249P variants that have not been considered to be responsible for NS phenotype. Further mutational and functional analyses will

elucidate the phenotypes of individuals with *LZTR1* variants and the functional consequences of these variants.

### Others

Chen *et al.*[35] identified a nonsense variant of *SPRY1*, a negative regulator of the RAS/ERK pathway as well as a missense variant of *MAP3K8* that encodes MAP kinase kinase kinase.[35] Further studies will be needed to clarify the pathogenetic significance of these variants.

## CARDIOVASCULAR ABNORMALITIES IN RASOPATHIES

Individuals with RASopathies often have cardiovascular abnormalities (Table 2). The frequency and type of cardiac involvement is different among the different disorders. Individuals with NS, Costello syndrome, CFC syndrome or NSML frequently develop cardiac abnormalities such as hypertrophic cardiomyopathy, pulmonic valve stenosis, septal defects and arrhythmia. More than 80% of individuals with NS have cardiovascular abnormalities.[26] Pulmonic valve stenosis is the most common cardiovascular abnormality in patients with NS.[28] Pulmonic valve stenosis is common (~70%) in individuals with *SOS1* and *PTPN11* mutations and is less frequent (~20%) in individuals with *RAF1* mutations.[53] Individuals with *RAF1* mutations and possibly also individuals with *RIT1* mutations frequently develop hypertrophic cardiomyopathy (~85 and ~50%, respectively).[29,35–37,53] In contrast, hypertrophic cardiomyopathy is less frequent in individuals with *SOS1* or *PTPN11* mutations.[53] NSML was previously referred to as LEOPARD syndrome (an acronym for its cardinal features of multiple *l*entigines, *e*lectrocardiographic conduction abnormalities, *o*cular hypertelorism, *p*ulmonary stenosis, *a*bnormal genitalia, *r*etardation of growth and sensorineural *d*eafness).[16] In contrast with the gain-of-function nature of the *PTPN11* mutations that have been identified in

**Table 2 Cardiovascular abnormalities associated with RASopathies**

| *Disorder* | *Genes* | *Clinical characteristics* | *Cardiovascular abnormalities* | *References for cardiac phenotypes* |
|---|---|---|---|---|
| Noonan syndrome (NS) | *PTPN11, SOS1, RAF1, RIT1, KRAS, NRAS, BRAF, RRAS* | Relative macrocephaly, distinctive facial features, short stature, mild developmental/cognitive impairment, webbed neck, cryptorchidism, Pectus excavatum, myeloproliferative disorder | More than 80% of individuals have cardiovascular abnormalities, including pulmonic valve stenosis (50–62%), hypertrophic cardiomyopathy (HCM) (~20%), atrial septal defects (6–10%) and unusual electrocardiographic patterns (50%). HCM is frequent in individuals with RAF1 mutations (~70%). HCM might be frequent in individuals with *RIT1* mutations. | 26,28,29,42 |
| NS with multiple lentigines (LEOPARD syndrome) | *PTPN11, RAF1, BRAF* | Multiple lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth and sensorineural deafness | Hypertrophic cardiomyopathy (~80% of individuals with cardiac defects), electrocardiographic abnormalities (73%), valvular defects (50%), pulmonary stenosis (~23%), coronary abnormalities (15%) | 57,58 |
| NS-like disorder with loose anagen hair | *SHOC2* | Macrocephaly, short stature with growth hormone deficiency, fine, sparse and easily pluckable hair, mild neurocognitive impairment | Approximately 80% of individuals have cardiovascular abnormalities, including pulmonary valve stenosis (39%), mitral valve dysplasia (31%), hypertrophic cardiomyopathy (27%) and septal defects (42%). | 20,59 |
| NS-like disorder | *CBL* | Variable. NS-like facial appearance, hyperpigmented skin lesions, microcephaly, developmental delay | Not frequently involved. Cardiomyopathy, arrhythmia, aortic or mitral valve anomalies | 21–23 |
| Costello syndrome | *HRAS* | Failure to thrive, distinctive facial features, feeding difficulties, short stature, curly hair, palmar keratosis, increased risk of malignant tumors (~10 to 15%) | Hypertrophic cardiomyopathy (~60%), congenital heart defects (~44%), valvular pulmonic stenosis (~22%) and atrial tachycardia (48%) | 61 |
| Cardiofaciocutaneous (CFC) syndrome | *BRAF*<br>*MAP2K1*<br>*MAP2K2*<br>*KRAS* | Failure to thrive, distinctive facial features, skin abnormalities including nevi, lentigines and palmar-plantar keratosis, curly hair, severe intellectual disability, seizure | 75% Of individuals with CFC syndrome have cardiovascular involvement. Pulmonary valvular stenosis (~45%), hypertrophic cardiomyopathy (~40%), septal defects, cardiac valve anomalies, arrhythmia, aortic dilatation | 63 |
| Neurofibromatosis type 1 (NF1) | *NF1* | Multiple café-au-lait spots, skin-fold freckling, neurofibromas, short stature, macrocephaly, Lisch nodules | NF1 vasculopathy (aneurysm, stenosis, arteriovenous malformation) in arteries of the kidney, heart and brain. Hypertension is frequently associated; 2.3% of individuals had cardiovascular malformations: valvular pulmonic stenosis, congenital heart defects and intracardiac neurofibromas | 5, 65–67 |
| NF1 like syndrome (Legius syndrome) | *SPRED1* | Multiple café-au-lait spots, skin-fold freckling, macrocephaly, learning disability, lipomas, NS-like face/characteristics | Not frequent. Pulmonary valve stenosis, mitral valve prolapse | 68 |
| Hereditary gingival fibromatosis | *SOS1* | Gingival fibromatosis | Not described | 24 |
| Capillary malformation–arteriovenous malformation | *RASA1* | Capillary malformation, arteriovenous malformation | Cardiac overload/failure is a potential complication. | 25,76,77 |

individuals with NS, the *PTPN11* mutations that have been identified in individuals with NSML have been shown to be catalytically inactive or dominant negative.[54–56] Mutations in *RAF1* and *BRAF* have less frequently been identified in individuals with NSML.[9,15] More than 80% of individuals with NSML present with heart defects; of these, hypertrophic cardiomyopathy occurs in 80%, electrocardiographic abnormalities in 73%, valvular defects in 50%, coronary abnormalities in 15% and septal defects in 1–5%.[57,58]

Individuals with NS-like disorder with loose anagen hair who have a common p.S2G mutation in *SHOC2* are characterized by a short stature that is associated with growth hormone deficiency, a Noonan-like facial appearance, mild neurodevelopmental delays and easily pluckable hair.[20] In two of the initial studies on this disorder, cardiac defects have been observed in 27 out of 33 (~80%) individuals.[20,59] Compared with individuals with NS, septal defects (~42%) and mitral valve anomalies (~31%) were more frequent.[20,59] In following case reports on individuals with the *SHOC2* p.S2G mutation, phenotypic variability was noted.[60]

Costello syndrome is a rare RASopathy that is characterized by distinctive facial features, including full lips, a large mouth and a full nasal tip; soft skin with deep palmer and planter creases, failure to thrive, mild to severe intellectual disability and increased risk of malignant tumors are also characteristics of these patients. Germline activating mutations in *HRAS* (G12S in ~80% of Costello syndrome patients) have been identified in individuals with Costello syndrome.[17] The majority of individuals with Costello syndrome have cardiac abnormalities; ~60% have hypertrophic cardiomyopathy, whereas ~44% have congenital heart defects that usually include nonprogressive pulmonary stenosis and ~48% present with atrial tachycardia.[61]

CFC syndrome shares many overlapping features with NS and Costello syndrome. Individuals with CFC syndrome have characteristic facial features, including high cranial vault, bitemporal constriction, hypoplastic supraorbital ridges, downslanting palpebral fissures, a depressed nasal bridge and posteriorly angulated ears with prominent helices.[62,63] Other clinical features include failure to thrive, hypotonia, motor delay, moderate intellectual disability and ectodermal abnormalities, such as sparse, friable hair, hyperkeratotic skin lesions and a generalized ichthyosis-like condition.[62,63] Germline mutations in *BRAF*, *MAP2K1/2* and *KRAS* have been identified in individuals with CFC syndrome.[18,19] In our previous cohort, *BRAF*, *MAP2K1/2* and *KRAS* mutations were identified in 68%, 23% and 9% of individuals, respectively.[64] *KRAS* mutations have also been identified in individuals with NS..[13] In CFC syndrome, ~75% of individuals have cardiovascular involvement, including pulmonic valve stenosis, hypertrophic cardiomyopathy and atrial septal defect in ~40%, ~30% and ~20% of individuals, respectively.[62]

NF1 is an autosomal dominant multisystem disorder affecting ~1 in 3000 newborn.[4] Clinical manifestations of NF1 include multiple café-au-lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, iris Lisch nodules and a distinctive osseous lesion such as sphenoid dysplasia or tibial pseudarthrosis. Legius syndrome is a NF1-like disorder, characterized by multiple café-au-lait macules, intertriginous freckling, lipomas, macrocephaly and learning disabilities without neurofibromas or other tumor manifestations.[8] Loss-of-function mutations in *SPRED1* have been identified in individuals with Legius syndrome.[8] Lin *et al.*[65] have reported that 54 out of 2322 (2.3%) individuals with NF1 had cardiovascular malformations. Of 54 individuals with cardiovascular abnormalities, flow defects resulting from abnormal embryonic intracardiac hemodynamics were observed in 43 (80%), pulmonic stenosis in 25 (58%) and aortic coarctation in 5 (9%).[65] Individuals with NF1 have been shown to have a wide range of vascular abnormalities.[66] Stenosis, aneurysms and occlusions of the major arteries and of arteries in the heart, brain and kidney were observed.[5,66] Hypertension is a relatively frequent manifestation.[67] Cardiac involvement is less frequent in individuals with Legius syndrome[68] and NS-like syndrome with CBL mutations.[21–23]

## CONCLUSIONS AND FUTURE PERSPECTIVE

The identification of the causative genes that underlie the RASopathies has facilitated molecular diagnosis of these disorders, enabled the evaluation of genotype–phenotype relationships and aided in the development of possible therapeutic approaches. Recent technical advances have led to the identification of novel genes that might be associated with RASopathies. Among these, a total of 32 individuals with *RIT1* mutations have been reported.[29,35–37] The clinical manifestations of *RIT1* mutation-positive individuals corresponded to those of NS. Rare variants of *RRAS*, *RASA2* and *SOS2* are probably associated with RASopathies because these molecules are functionally related to the RAS/ERK pathway. Further analyses of additional cohorts and of the functional roles of *A2ML1* and *LZTR1* will be required to conclude that these rare variants are associated with RASopathy pathogenesis. The identification of RASopathy-related genes will also provide new insights into the biology of the RAS/MAPK signaling pathway.

A variety of cardiovascular abnormalities have been associated with individuals who are affected by RASopathies. The appropriate treatment of these cardiovascular abnormalities leads to better prognoses for patients with these disorders. Inhibitors of the RAS/MAPK signaling cascade may offer a means of therapeutically treating disorders that involve dysregulation of the RAS/MAPK pathway.[69] The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors have been used in clinical trials to enhance cognitive function in individuals with NF1.[70] An open-label study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of a MEK inhibitor, MEK162 (Novartis), in adults with NS who also have hypertrophic cardiomyopathy is now recruiting (ClinicalTrials.gov identifier: NCT01556568). Indeed, MEK inhibitors have been shown to ameliorate the phenotype of knock-in mouse models for NS (mutations in *Sos1* and *Raf1*)[71,72] and CFC syndrome (*Braf* mutation),[73] suggesting that the phenotypes that are produced by RASopathies can be ameliorated by manipulating RAS/MAPK activity. An inhibitor of mammalian target of rapamycin has been shown to reverse heart defects in both a mouse model of[74] and in an individual with NSML.[75] Histone demethylase inhibitors that might not be directly associated with the RAS/ERK pathway have also been shown to ameliorate the phenotype of a mouse model of CFC syndrome.[73] Further studies will explore the pathogenetic mechanisms behind and therapeutic approaches for RASopathies.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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