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Ras/MAPK syndromes and childhood hemato-oncological diseases

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Abstract Noonan syndrome (NS) is an autosomal-dominant disease characterized by distinctive facial features, webbed neck, cardiac anomalies, short stature and cryptorchidism. NS exhibits phenotypic overlap with Costello syndrome and cardio-facio-cutaneous (CFC) syndrome. Germline mutations of genes encoding proteins in the RAS/mitogen-activated protein kinase (MAPK) pathway cause NS and related disorders. Germline mutations in *PTPN11*, *KRAS*, *SOS1*, *RAF1*, and *NRAS* have been identified in 60–80 % of NS patients. Germline mutations in *HRAS* have been identified in patients with Costello syndrome and mutations in *KRAS, BRAF,* and *MAP2K1/2* (MEK1/2) have been identified in patients with CFC syndrome. Recently, mutations in *SHOC2* and *CBL* have been identified in patients with Noonan-like syndrome. It has been suggested that these syndromes be comprehensively termed RAS/MAPK syndromes, or RASopathies. Molecular analysis is beneficial for the confirmation of clinical diagnoses and follow-up with patients using a tumor-screening protocol, as patients with NS and related disorders have an increased risk of developing tumors. In this review, we summarize the genetic mutations, clinical manifestations, associations with malignant tumors, and possible therapeutic approaches for these disorders.

Keywords RAS/MAPK signaling pathway · RASopathies · Oncogene · RAS · RAF · MEK

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Introduction

Noonan syndrome (NS, MIM 163950) was first described by Jacqueline Noonan, a pediatric cardiologist, in 1962. NS is an autosomal dominant disorder characterized by short stature, facial dysmorphism and congenital heart defects. The distinctive facial features that manifest in NS include a webbed or short neck, hypertelorism, downslanting palpebral fissures, ptosis and low-set, posteriorly rotated ears [1, 2]. Congenital heart defects, including pulmonary valve stenosis, occur in 50–80 % of individuals. Hypertrophic cardiomyopathy is observed in 20 % of affected individuals. Other clinical manifestations include cryptorchidism, bleeding tendency, mild intellectual disability, deafness, and hydrops fetalis. The incidence of this syndrome is estimated to be between 1 in 1,000 and 1 in 2,500 live births [3]. NS is known to be associated with juvenile myelomonocytic leukemia (JMML), a myeloproliferative disorder characterized by excessive production of myelomonocytic cells [1].

The phenotypic features of NS are similar to those of Costello and cardio-facio-cutaneous (CFC) syndromes. Costello syndrome (MIM 218040) was originally described by Costello in 1971 [4] and explored further by the same author in 1977 [5]. Patients with Costello syndrome have distinctive facial characteristics, including full lips, a large mouth, and a full nasal tip, and exhibit mental retardation, high birth weight, neonatal feeding problems, curly hair, nasal papillomata, and soft skin with deep palmar and plantar creases [6]. Cardiac defects include hypertrophic cardiomyopathy, congenital heart defects and arrhythmia. Children and young adults with Costello syndrome have an increased risk of malignancy, which can be as high as approximately 17 % [7].

CFC syndrome (MIM 115150) was first described in 1986 [8], and the question of whether CFC and NS are

distinct disorders or different phenotypes of the same condition is controversial. CFC syndrome is characterized by distinctive facial features, mental retardation, heart defects (pulmonic stenosis, atrial septal defect, and hypertrophic cardiomyopathy) and ectodermal abnormalities, such as sparse, friable hair, hyperkeratotic skin lesions and a generalized ichthyosis-like condition [9]. Typical facial characteristics include a high forehead with bitemporal constriction, hypoplastic supraorbital ridges, downslanting palpebral fissures, a depressed nasal bridge and posteriorly angulated ears with prominent helices.

NS with multiple lentigines was formerly referred to as LEOPARD syndrome. LEOPARD is an acronym for the cardinal features of the syndrome, which include multiple Lentigines, Electrocardiographic conduction abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retardation of growth and sensorineural Deafness [10].

RAS GTPases are essential mediators in signaling pathways that convey extracellular stimuli from cell surface receptors to the cell. The Ras subfamily consists of classical Harvey-RAS (HRAS), Kirsten-RAS (KRAS) and neuroblastoma RAS (NRAS). Other distinct members include R-RAS, TC21 (R-RAS2), M-RAS (R-RAS3), Rap1A, Rap1B, Rap2A, Rap2B, RalA and RalB [11]. The RAS/mitogen-activated protein kinase (MAPK) pathway is an essential signaling pathway that controls cell proliferation, differentiation and survival. Recent studies have revealed that dysregulation of the RAS/MAPK pathway causes clinically overlapping genetic disorders, including NS, Costello syndrome, CFC syndrome, NS with multiple lentigines, neurofibromatosis type I, and Legius syndrome [6, 12]. This review outlines the molecular aspects, clinical manifestations, association with malignant tumors, and possible treatments of NS, Costello syndrome and CFC syndrome.

Genes and mutations that underlie genetic syndromes involving dysregulation of the RAS/MAPK signaling pathway (Table 1)

Background

In 1994, linkage analysis of a large family with NS definitively established the first NS locus, which was defined as chromosomal bands 12q22-qter [13, 14]. In 2001, Tartaglia et al. [15] identified missense mutations in *PTPN11*, which encodes the tyrosine phosphatase SHP-2, in individuals with NS. Gain-of-function mutations in *PTPN11* have been identified in approximately 50 % of individuals with clinically diagnosed NS [16–18] (Fig. 1). In contrast, loss-of-function or dominant negative mutations in *PTPN11* have been reported in patients with NS with multiple lentigines [10]. In 2005, we performed candidate gene analysis of proteins in the RAS/MAPK cascade and discovered germline mutations in *HRAS* in patients with Costello syndrome [19]. Subsequently, mutations in *KRAS*, *BRAF*, and *MAP2K1/2* have been identified in patients with CFC syndrome [20, 21], and mutations in *KRAS, SOS1, RAF1*, and *NRAS* have been identified in patients with NS [22–27] (Fig. 1). Recently, mutations in *SHOC2* [28] and *CBL* [29–31] have been identified in NS-like syndromes. These findings indicate that RAS and molecules downstream of RAS play essential roles in human development. It has been suggested that these syndromes be comprehensively termed 'RAS/MAPK syndromes' or 'RASopathies' [6, 12].

Table 1 Summary of RAS/MAPK syndromes

Disorder	MIM	Inheritance	Gene(s) mutated
NS	163950	AD	PTPN11, KRAS, SOS1, RAF1, NRAS
Noonan-like disorder with loose anagen hair	607721	AD	SHOC2
NS-like disorder	613563	AD	CBL
Costello syndrome	218040	AD	HRAS
Cardio-facio-cutaneous (CFC) syndrome	115150	AD	BRAF, MEK1, MEK2, KRAS
Neurofibromatosis type I	162200	AD	NF1
NF-1 like syndrome (Legius syndrome)	611431	AD	SPRED1
NS with multiple lentigines (LEOPARD syndrome)	151100	AD	PTPN11, RAF1, BRAF
Hereditary gingival fibromatosis	135300	AD	SOS1
Capillary malformation-arteriovenous malformation	608354	AD	RASA1

MIM Mendelian inheritance in man, *AD* autosomal dominant, *LEOPARD* multiple Lentigines, Electrocardiographic conduction abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retardation of growth and sensorineural Deafness

Genes and mutations

PTPN11

SHP-2, the product of *PTPN11*, is a widely expressed cytoplasmic tyrosine phosphatase that has been implicated in signal transduction pathways elicited by growth factors, cytokines, hormones and the extracellular matrix [32]. SHP-2 comprises a tandem array of two SH2 domains at its N-terminus, a catalytic domain in the middle, and a C-terminal domain that contains tyrosine phosphorylation

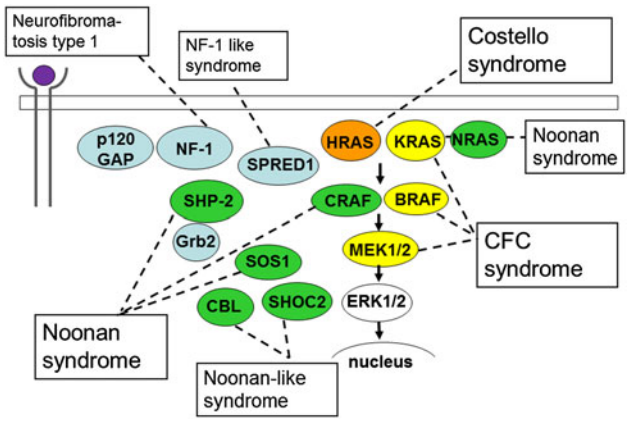

Fig. 1 RAS/MAPK cascade and disorders involving germline mutations of related genes

sites. Most mutations identified in NS were clustered in exons 3, 4, 7, 8, 12 and 13. Y63C, Q79R, N308D, and N308S were the most common mutations [6, 33]. T73I has been frequently identified in NS patients with JMML. Specific mutations (Y279C, A461T, G464A, T468M, D498W and Q510P) have been identified in NS with multiple lentigines.

Mutations that have been previously identified in NS are located in the interacting face of the N-SH2 domain and phosphatase domain, suggesting that they are gain-of-function mutations that enhance phosphatase activity. SHP-2 mutants associated with leukemia were more catalytically active than mutants identified in NS patients, suggesting that a high level of SHP-2 activation is associated with neoplastic diseases, whereas a lower level of SHP-2 activation causes NS [17, 34–36]. Mutations identified in NS with multiple lentigines have been shown to be catalytically inactive or dominant negative [36–38].

SOS1

SOS1 is a ubiquitously expressed guanine nucleotide exchange factor (GEF) that is responsible for the activation of RAS proteins by catalyzing GDP/GTP exchange. Mutations in *SOS1* have been identified in 8–14 % of patients with NS [25, 27], and the biochemical characterization of SOS1 mutants has indicated enhanced protein function and increased downstream signaling. Compared with other NS patients, the incidence of short stature and intellectual disability is lower in patients who tested positive for an *SOS1* mutation [39].

RAF1

RAF1 is a member of the RAS serine–threonine kinase family. Mutations in *RAF1* have been identified in 3–17 % of patients with NS and a small number of patients with NS with multiple lentigines [23, 24]. Mutations identified in NS were clustered in conserved region (CR) 2, which contains an inhibitory phosphorylation site (serine at position 259; S259). RAF1 mutations located in the CR2 domain caused a decrease in the phosphorylation of S259, leading to partial ERK activation [39]. Notably, 70 % of patients with *RAF1* mutations exhibit hypertrophic cardiomyopathy [23, 24, 39, 40].

HRAS

Individuals with *HRAS* mutations are diagnosed as having Costello syndrome, and heterozygous *HRAS* mutations have been identified in more than 90 % of patients with this syndrome [6, 19]. Germline mutations are clustered in codons 12 and 13, and the G12S mutation is the most frequent (80 %). *HRAS* germline mutations occur de novo. Somatic mosaicism for the G12S mutation has been reported in three individuals with clinical findings suggestive for Costello syndrome [41].

KRAS

Individuals with *KRAS* mutations exhibit variable phenotypes and are diagnosed with NS or CFC syndrome [20, 26, 42]. Mutations in codons 12, 13, and 61, which are frequent in somatic cancers, have rarely been identified as germline mutations, which is in contrast with *HRAS* germline mutations. V14I is a frequent mutation in NS, and D153V mutation has been identified in patients with NS or CFC syndrome [6]. The effects of *KRAS* germline mutations on the downstream pathway are less pronounced than the effects of somatic mutations [20, 26]

BRAF and MAP2K1/2 (MEK1/2)

Mutations in *BRAF* and *MAP2K1/2* have been identified in patients with CFC syndrome [20, 21]. *BRAF* mutations have also been identified in patients with a phenotype of NS with multiple lentigines. Somatic mutations in *BRAF* have been identified in 7 % of all cancers, including human malignant melanoma and colorectal cancer [43]. V600E mutation in the activation segment in the kinase domain (CR3) has been frequently identified in somatic cancers (>90 %). Germline mutations were clustered in the cysteine-rich domain (CR1 domain) and kinase domain. The distribution of the mutations identified in CFC syndrome partially overlapped with that of the mutations identified in cancers. Q257R and E501G are frequent mutations in CFC syndrome [6]. Germline mutations in *MAP2K1/2* are clustered in exons 2 and 3. ERK phosphorylation was

enhanced in cells transfected with mutant MEK1 or MEK2 [21]. Affected individuals harboring *BRAF* and *MAP2K1/2* mutations have the disorder as a result of a de novo mutation. One family transmitting an autosomal dominant germline mutation in *MAP2K2* has been reported [44].

SHOC2

SHOC2 is homologous to *soc2*, a gene discovered in *Caenorhabditis elegans*. The *soc2* gene encodes leucine-rich repeats [45] and acts as a positive modulator of the RAS/MAPK pathway [46]. In 2009, a gain-of-function missense mutation in *SHOC2*, c.4A > G (p.S2G), was identified in patients with Noonan-like syndrome with loose anagen hair [28]. The clinical features associated with the S2G mutation are distinct from those associated with NS and include a high frequency of loose anagen hair, more severe intellectual disabilities, skin abnormalities, and a hypernasal voice [28, 47]. Wild-type SHOC2 is localized in the nucleus and cytoplasm, while the mutant protein (S2G) promoted aberrant N-myristoylation, localized to the plasma membrane and resulted in ERK activation [28].

NRAS

A germline mutation in *NRAS* has been reported in a patient with autoimmune lymphoproliferative syndrome. Germline mutations in *NRAS* have been reported in four of 917 NS patients (0.4 %) who were negative for previously known mutations [22]. The amino acid changes identified in NS patients were I24N, P34L, T50I and G60E [22, 48, 49]. Cells carrying T50I and G60E mutations and oncogenic G12V displayed enhanced MEK and ERK phosphorylation when serum was added to the growth medium [22].

CBL

Casitas B-cell lymphoma (CBL) is the cellular homolog of the v-Cbl transforming gene of the Cas NS-1 murine leukemia virus. CBL functions primarily as an E3 ubiquitin ligase and is responsible for the intracellular transport and degradation of a large number of proteins. The majority of *CBL* somatic mutations have been reported in myelodysplastic syndromes/myeloproliferative disorders, including chronic myelomonocytic, juvenile myelomonocytic and atypical chronic myeloid leukemias. Germline mutations in *CBL* have been identified in JMML patients who displayed a variable combination of dysmorphic features reminiscent of the facial gestalt of NS [29–31].

Association of tumors and hematologic malignancies in patients with germline mutations in genes within the RAS/MAPK pathway (Table 2)

Somatic mutations in genes in the RAS pathway, including *PTPN11*, *HRAS*, *KRAS*, *NRAS*, *BRAF* and *CBL*, have been identified in a variety of solid tumors and hematologic malignancies. NS and related disorders are known to cause a predisposition to cancer. The precise frequency of tumor predisposition in mutation-positive patients remains unknown.

It has been reported that the *PTPN11* mutations in patients with NS are frequently associated with hematologic malignancies, including acute lymphoblastic leukemia and JMML. The frequency of association with tumors remains unknown. In a summary of the literature, Kratz et al. [50] reported that 45 of 1,151 patients (3.9 %) with NS (mutation status unknown) developed cancer; of these, eight patients presented with neuroblastoma, and eight presented with acute lymphoblastic leukemia. The other cancers identified included six gliomas, six rhabdomyosarcoma, three acute myeloid leukemias, three testicular cancers, two non-Hodgkin lymphomas and two colon cancers. *PTPN11* mutations in patients with NS have been shown to be associated with myeloproliferative disorder, and with a benign course in 40 % of such patients, and an aggressive course in 15 % [50]. It remains unknown why gain-of-function mutations in *PTPN11* enhance the proliferation of specific lineages in hematologic malignancies.

Approximately, 10–15 % of patients with Costello syndrome develop malignant tumors, including rhabdomyosarcoma, neuroblastoma (in infants) and transitional cell carcinoma of the bladder (in adolescents and young adults) [6]. A tumor screening protocol for patients with Costello syndrome has been proposed [7]. Notably, *HRAS* mutations were originally identified in bladder carcinoma cell lines. It remains unknown why patients with *HRAS* germline mutations develop bladder carcinomas.

Little attention had been given to the development of tumors in patients with CFC syndrome until molecular analysis became available. Two CFC patients with *BRAF* mutations were reported to have developed acute lymphoblastic leukemia [20, 51], and one CFC patient with a *BRAF* mutation was reported to have developed non-Hodgkin lymphoma [52]. Somatic *BRAF* mutations in hematologic malignancies do not occur frequently, but they are substantially reported. Recently, *BRAF* mutations have been identified in Langerhans cell histiocytosis [53]. It is possible that the role of BRAF in hematologic malignancies may indicate that BRAF plays roles in other malignancies beyond solid tumors. As for *MAP2K1/2*, one patient with a *MAP2K1* mutation developed hepatoblastoma [54].

Table 2 Disorders involving germline and somatic mutations in genes of the RAS/MAPK cascade

Gene	Germline mutations		Somatic mutations
	Disorders	Associated tumors	Tumors and related disorders
PTPN11	NS, NS with multiple lentigines (LEOPARD syndrome)	JMML, myeloproliferative disorders [50]	JMML, AML, ALL, MDS, CML, solid tumors
SOS1	NS	Rhabdomyosarcoma (1 pt), Sertoli cell tumor (1 pt), granular cell tumors (1 pt) [55]	Rare (astrocytoma, lung cancer)
RAF1	NS, NS with multiple lentigines (LEOPARD syndrome)	–	Rare (AML, lung cancer, ovarian cancer, colon cancer)
SHOC2	Noonan-like disorder with loose anagen hair	–	–
NRAS	NS	–	Hematologic malignancies
CBL	NS-like disorder	JMML [30, 31]	JMML, CML, AML, MDS, lung cancer
HRAS	Costello syndrome	Rhabdomyosarcoma, neuroblastoma, bladder carcinoma, papillomata	Bladder carcinoma
KRAS	CFC syndrome, NS	JMML (1 pt) [26]	Pancreatic tumor, colon cancer, lung cancer, RAS-associated ALPS-like disease [63]
BRAF	CFC syndrome NS	ALL (2 pts) [20, 51], non-Hodgkin lymphoma (1 pt) [52]	Malignant melanoma, colon cancer, thyroid cancer, Langerhans cell histiocytosis [53]
MEK1	CFC syndrome	Hepatoblastoma (1 pt) [54]	–
MEK2	CFC syndrome	–	–

JMML juvenile myelomonocytic leukemia, *ALL* acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *CML* chronic myelogenous leukemia, *MDS* myelodysplastic syndromes, *ALPS* autoimmune lymphoproliferative syndrome

Germline mutations in *CBL* have been identified in JMML patients who displayed a variable combination of dysmorphic features reminiscent of the facial gestalt of NS. Facial appearances, psychomotor development, head circumference and skin abnormalities should be carefully observed in children with hematologic malignancies. A patient with a *KRAS* mutation who developed JMML has been reported [26]. It has been reported that three patients with *SOS1* germline mutations developed rhabdomyosarcoma (one patient), Sertoli cell tumors (one patient), and granular cell tumors (one patient) [55]. As far as we know, tumor association has not been reported in individuals with germline mutations in *SHOC2*, *NRAS*, and *RAF1*.

The natural history and predisposition for hematologic malignancies and solid tumors in adults with RAS/MAPK syndromes have not been clarified. We conducted a nationwide epidemiologic study on patients with Costello and CFC syndromes in 2009 [56]. The results showed that the total number of patients with Costello and CFC syndrome in Japan was estimated to be 99 (95 % confidence interval, 77–120) and 157 (95 % confidence interval, 86–229), respectively. An evaluation of 15 adult patients (18–32 years of age) revealed that one had recurrent bladder papillomata and another had multiple gallbladder polyps and a renal angioma. None of the examined patients developed malignant tumors. Twelve of 15 adult patients had moderate to severe mental retardation, but eleven live at home, and 10 can walk independently, suggesting that a portion of adult patients may be unrecognized and the number of adult patients is likely underestimated. Therefore, the prognosis, including the frequency of malignant tumors, remains to be elucidated in adults with germline mutations in *RAS* and *RAF*.

Conclusions

The identification of the causative genes underlying NS and related disorders has facilitated the molecular diagnosis of these disorders, allowed the evaluation of the genotype–phenotype relationship and helped develop possible therapeutic approaches. In approximately 10–30 % of patients with RAS/MAPK syndromes, no mutations were identified. The introduction of exome sequencing will lead to the identification of novel genes involved in these disorders.

The regulation and inhibition of the RAS/MAPK pathway have been well studied in cancer research. Inhibitors of the RAS/MAPK cascade may provide opportunities to therapeutically treat disorders involving dysregulation of the RAS/MAPK pathway [57]. Indeed, MEK inhibitors ameliorated the phenotype of mice models for NS (mutations in *SOS1* and *RAF1*) [58, 59] and Costello syndrome (mutation in *HRAS*) [60]. An inhibitor of mTOR has been shown to reverse heart defects in a mouse model for NS with multiple lentigines [61]. HMG-CoA reductase inhibitors have been used in clinical trials to treat cognitive

function in individuals with NF1 [62]. These results suggest that the phenotypes in RAS/MAPK syndromes can be ameliorated by the manipulation of RAS/MAPK activity.

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