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Noonan syndrome and clinically related disorders

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Practice Points

- Noonan syndrome (NS) is a relatively common, clinically variable disorder transmitted as an autosomal dominant trait with a high proportion of sporadic cases due to de novo mutations.
- Clinical diagnosis of NS is based on the variable association of distinctive facial dysmorphism, congenital cardiac defects and/or hypertrophic cardiomyopathy, postnatally reduced growth, thorax anomalies, variable cognitive deficit, cryptorchidism, and lymphatic dysplasia.
- NS is genetically heterogeneous, and mutations in PTPN11, SOS1, RAF1, KRAS, NRAS and BRAF account for approximately 75% of cases. These genes encode for functionally related proteins implicated in a common signal transduction pathway. Clinically relevant genotypephenotype correlations have been documented.
- There are a number of developmental disorders clinically (and genetically) related to NS. Among them, LEOPARD syndrome (PTPN11, RAF1 and BRAF), Noonan-like syndrome with loose anagen hair (SHOC2), neurofibromatosis-Noonan syndrome (NF1), cardiofaciocutaneous syndrome (BRAF, KRAS, MEK1 and MEK2), and "CBL mutation-associated" syndrome (CBL) are the most close.
- Molecular diagnosis is available and may help to make the diagnosis, particularly in infants and young children, and may aid in defining risks for particular morbidities.
- Clinical management focuses on cardiological treatment and follow-up, monitoring of growth and development, and symptomatic interventions as required.
- Short stature is tentatively treated by use of pharmacological doses of recombinant GH. In many Countries, availability of this therapy is currently restricted to patients with documented GH deficiency or secretory dysfunction.

Research Agenda

- The genetic basis in 25 % of NS cases and in a still undetermined fraction of patients with clinical features partially overlapping or suggestive of NS is still unknown.
- The biological consequences of mutations in identified disease genes at the cellular level have to be elucidated more precisely.
- Studies directed to develop therapeutic interventions to ameliorate postnatal issues of this disease, such as hypertrophic cardiomyopathy, reduced growth, and cognitive deficits are required.
- Relevant studies on the long-term efficacy and safety of growth promoting therapy with recombinant GH are missing.

Abstract

Noonan syndrome is a relatively common, clinically variable developmental disorder. Cardinal features include postnatally reduced growth, distinctive facial dysmorphism, congenital heart defects and hypertrophic cardiomyopathy, variable cognitive deficit and skeletal, ectodermal and hematologic anomalies. Noonan syndrome is transmitted as an autosomal dominant trait, and is genetically heterogeneous. So far, heterozygous mutations in nine genes (PTPN11, SOS1, KRAS, NRAS, RAF1, BRAF, SHOC2, MEK1 and CBL) have been documented to underlie this disorder or clinically related phenotypes. Based on these recent discoveries, the diagnosis can now be confirmed molecularly in approximately 75% of affected individuals. Affected genes encode for proteins participating in the RAS-mitogen-activated protein kinases (MAPK) signal transduction pathway, which is implicated in several developmental processes controlling morphology determination, organogenesis, synaptic plasticity and growth. Here, we provide an overview of clinical aspects of this disorder and closely related conditions, the molecular mechanisms underlying pathogenesis, and major genotype-phenotype correlations.

Keywords

Noonan syndrome; LEOPARD syndrome; Noonan-like syndrome with loose anagen hair; CBL mutation-associated syndrome; RAS signaling; pathogenetic mechanisms; diagnosis; patient management; genotype-phenotype correlations

Introduction

Noonan syndrome (NS, OMIM 163950) is the eponymous name for the disorder described by the pediatric cardiologist Jacqueline Noonan more than forty years ago [1]. She based the description of this putatively novel syndrome on observations made in nine patients with pulmonic stenosis (PS), a distinctive dysmorphic facial appearance with hypertelorism, ptosis and low-set ears, webbed neck, and chest deformities. Several male patients also had cryptorchidism. NS is thought to be relatively common, although its prevalence has not been determined accurately to date. Most authors cite the figure of 1 in 1,000-2,500 live births firstly reported by Nora and colleagues [2].

NS is a Mendelian trait transmitted in an autosomal dominant manner, and as observed for other dominant disorders, a significant, but not precisely determined, percentage of cases is due to de novo mutations. NS is genetically heterogeneous, and nine genes (PTPN11, SOS1, KRAS, NRAS, RAF1, BRAF, SHOC2, MEK1 and CBL) have been causally linked to this trait or closely related conditions, including LEOPARD syndrome (LS; OMIM 151100), Noonan-like syndrome with loose anagen hair (NS/LAH, OMIM 607721), and the recently recognized “CBL mutation-associated” syndrome). Mutations in a subgroup of those genes as well as in genes encoding for signal transducers participating in the same pathway (HRAS, KRAS, NF1, BRAF, SPRED1, MEK1 and MEK2) have been implicated in other related disorders (i.e., Costello syndrome, cardiofaciocutaneous syndrome, neurofibromatiosis type 1, including neurofibromatosis-Noonan syndrome, and Legius syndrome). Based on this shared pathogenetic mechanism and clinical overlap, these diseases have been grouped into a single family, which has been termed the neurocardiofacialcutaneous syndrome family (or, alternatively, the RAS-opathies) [3,4]. In this review, we outline the clinical aspects and molecular genetics of NS and clinically closely phenotypes.

Noonan syndrome: Clinical features and diagnosis

NS is a disorder characterized by postnatally reduced growth, distinctive facial dysmorphic features, congenital heart defects (CHD), hypertrophic cardiomyopathy (HCM), skeletal anomalies, and webbing of the neck. Other relatively common features are bleeding diathesis, ectodermal anomalies, lymphatic dysplasias, cryptorchidism, and cognitive deficits [5-7]. NS is characterized by marked phenotypic variability, which can be explained, in part, by the underlying molecular lesions. The diagnosis of NS depends primarily on the clinical features (Table 1), although the prevalence of the characteristic features among affected individuals has not been rigorously assessed and depends on the patient's age [8]. In newborns, the facial features may be less apparent and length is typically normal but lymphedema and excess nuchal folds may be present. With time, several features become more obvious, including facial dysmorphism, pectus deformities, and reduced growth. HCM may also develop during the first few years of life. The facial features can become more difficult to detect in later adolescence and adulthood. While comprehensive scoring systems were developed to aid in making the diagnosis clinically [7,9], most clinicians continue to use a more subjective assessment. Genetic testing is now available to confirm or make the diagnosis. Molecular screening efforts have shown that approximately 75% of individuals with a diagnosis of NS harbor a mutation in PTPN11, SOS1, KRAS, NRAS, RAF1, BRAF or MEK1, but this percentage is dependent on the diagnostic criteria used to make the diagnosis. As additional NS disease gene(s) remain to be discovered, a failure to identify a mutation does not exclude the diagnosis. The diagnosis of NS can be suspected prenatally when a cystic hygroma or nuchal lucency is detected with fetal ultrasonography, particularly when associated with additional disease features, such as CHD. Since birth height and weight are usually normal in NS, parameters of fetal growth are unlikely to be helpful in making the diagnosis.

Cardiac defects and short stature are the major causes for patients with NS requiring medical attention. While birth length is typically normal, growth parameters usually drop below the 3rd centile during the first years of life. Since there is often some attenuation and/or delay of the pubertal growth spurt, the prevalence of short stature in NS is highest during the age of normal puberty. This is accompagnied by a delay in bone age. Many patients show some catch-up growth in their late teens. This has to be taken into account for therapeutic decisions. While there have been reports on growth hormone (GH) deficiency, neurosecretory dysfunction, or GH resistance in NS [10-12], a consistent pattern of abnormal GH secretion or action has not been shown so far, and it seems unlikely that there is a simple link between GH and the growth deficits in NS. GH treatment in NS is still a matter of debate. Published surveys are difficult to compare due to the heterogeneous protocols and outcome criteria used [13-16]. It seems that GH therapy may lead to a height gain of up to 10 cm depending on the age of initiation and the duration of treatment, and the indication for GH treatment is broadly accepted in the fraction of NS patients with GH deficiency. It should be noted that adult stature is normal in nearly 50% of cases even without intervention with growth hormone therapy [17].

Facial features of NS include high forehead, hypertelorism, downslanting palpebral fissures, epicantal folds, ptosis, low-set and/or posteriorly rotated ears (Figure 1). Besides the short and/or webbed neck, a low posterior hairline commonly occurs. Affected individuals exhibit a wide spectrum of cardiac disease [18,19]. PS, septal defects, and HCM occur most commonly, but other lesions are also observed. Feeding problems are noted in the majority of affected infants and can cause failure to thrive [20]. While feeding difficulties resolve in most patients by around age 18 months, the inadequacy in oral intake may be severe enough to necessitate placement of gastrostomy tubes in some. Developmental delay and learning problems are quite common. Some motor delay can be attributed to the hypotonia often observed in affected infants. An increased prevalence of attention deficit/hyperactivity

disorder and frank mental retardation are also observed. Available data indicate that the heterogeneity in cognitive abilities observed in NS is at least partially ascribed to the specifics of the causative gene mutation [21,22]. Skeletal anomalies most frequently consist of pectus deformities, cubitus valgus, vertebral defects, and scoliosis.

Juvenile myelomonocytic leukemia (JMML, OMIM 607785), a rare myeloproliferative disorder of childhood [23], arises at increased prevalence in NS although it affects only a small percentage of patients [24]. The myeloproliferative disorder in children with NS may regress without treatment, follow an aggressive clinical course or evolve to acute myeloid leukemia. This condition is associated with a narrow spectrum of mutations affecting the PTPN11 gene (see below) [24,25], but it also can be associated with certain germline KRAS mutations [26]. NS is rarely accompanied by multiple benign giant cell lesions (MGCLs) of the jaw and/or other bone/soft tissues. This finding was originally introduced as a distinct nosologic entity named Noonan-like/multiple giant cell lesion syndrome (NL/MGCLS, OMIM 163955) [27]. Now, it is apparent that MGCLs are an associated feature of NS and that NL/MGCLS is not a separate disorder. This view is supported by the observation that MGCLs has been documented to occur in different conditions of the NCFC syndrome family, and to be caused by mutations affecting PTPN11, SOS1, BRAF, and MEK1 genes previously reported in subjects with those syndromes without any evidence of bony involvement [28-31]. Based on these considerations, the use of NL/MGCLS should be avoided.

Disorders clinically related to Noonan syndrome

LEOPARD syndrome—LEOPARD syndrome (LS, OMIM 151100) is an autosomal dominant trait that overlaps phenotypically with NS (Figure 2). It is also allelic with NS, with a restricted spectrum of mutations in PTPN11 accounting for the vast majority of affected individuals [32,33]. In a small proportion of cases, LS has been causally linked to mutations in RAF1 or BRAF [34-36]. The acronymic name refers to the major features: Lentigines, ECG conduction abnormalities, Ocular hypertelorism, Pulmonic stenosis, Abnormal genitalia, Retardation of growth, and sensorineural Deafness [37]. Similar to NS, there are age-dependent aspects of the phenotype. Craniofacial dysmorphism is similar to that of NS but is usually milder [38], and the neck is short but usually not webbed. Multiple lentigines, which are flat, black-brown macules, are dispersed primarily on the face, neck, and upper part of the trunk, but sparing the mucosae. In general, lentigines appear at the age of 4-5 years and increase until puberty into the thousands. Café-au-lait spots are also observed, alone or in association with lentigines, in up to 70-80% of the patients [38], and usually precede the appearance of lentigines, being present from the first months of life. Growth retardation (height below the 3rd centile) is observed in 25% of affected individuals, and final height in 85% of subjects is below the 25th centile. Approximately half of affected individuals have heart defects, which are similar to those recurring in NS but recur with different frequencies. ECG anomalies, progressive conduction defects and HCM are the most frequent features. Of note, HCM is detected in up to 80% of LS patients with heart defects, most commonly appearing during infancy. It is progressive during childhood, and paralleled by the appearance of lentigines. Diagnostic criteria for LS have been outlined [39]. A clinical diagnosis is not always feasible, particularly in young patients with no lentigines, due to the overlap with NS and neurofibromatosis-Noonan syndrome (see below). In these patients, the clinical differentiation from NS may be difficult during infancy and early childhood. The occurrence of distinct associated signs, including HCM, sensorineural deafness, and café-au-lait spots represents an important diagnostic handle in these young patients [38]. Sarkozy and colleagues [40] provide an updated review of the clinical and molecular genetics aspects of this disorder.

Noonan syndrome-like with loose anagen hair—Subjects with Noonan syndrome-like with loose anagen hair (NS/LAH; OMIM 607721) show features that are at first view reminiscent of NS (Figure 3). The phenotype of these subjects, however, is notable for reduced growth often associated with proven GH deficiency, more significant cognitive deficits, distinctive behavior, and easily pluckable, sparse, thin, slow growing hair in the anagen phase but lacking an inner and outer root sheaths [41,42]. Such hair anomalies fit a well-known condition termed loose anagen hair (LAH) syndrome, which is clinically heterogeneous and has been reported to occur typically in young children and, in most cases, spontaneously improves clinically as early as adolescence [43]. In these subjects, LAH can be confirmed by microscopic examination of plucked hairs. Most affected individuals exhibit hairless and darkly pigmented skin with eczema or ichthyosis, and a tendency to pruritus. Ectodermal anomalies also include sparse eyebrows and dystrophic or thin nails. The voice is characteristically hoarse or hypernasal. Cardiac anomalies are observed in the majority of cases, with dysplasia of the mitral valve and septal defects significantly overrepresented compared with the general NS population. So far, NS/LAH appears to be genetically homogeneous, as all affected individuals share the same 4A>G missense change (Ser2Gly) in SHOC2, which encodes a scaffold protein required for the efficient transmission of information from RAS to the MAPK cascade [42].

CBL-mutation associated syndrome—Three independent studies recently reported that germline heterozygous mutations in the CBL gene underlie a previously unrecognized condition with features fitting or partially overlapping NS in some individuals (Figure 4) [44-46]. Accordingly, these lesions appear to be associated with a strikingly variable phenotype, with clinical features that might be quite subtle in some subjects. Relatively common features occurring in CBL mutation positive subjects include include variable developmental delay and reduced growth, facial dysmorphism, and café-au-lait spots. Germline CBL mutations are likely to account for a small portion of subjects with features fitting NS (< 1%), but might be more common among subjects with clinical features partially overlapping NS or a phenotype that might be suggestive of this disorder [44]. This condition confers predisposition to JMML during childhood [45,46].

Neurofibromatosis type 1 and related phenotypes—Neurofibromatosis type 1 (NF1; OMIM 162200) is one of the most common autosomal dominant diseases (1 in 3000-4000 live births). Its main clinical characteristics include cutaneous, subcutaneous and/or plexiform neurofibromas, café-au-lait spots, axillary and/or inguinal freckling, Lisch nodules in the iris, skeletal deformities, vascular defects, learning disabilities and behavioral problems, short stature, macrocephaly and a predisposition for developing benign and malignant neoplasias [47,48]. The diagnosis of NF1 is based on clinical criteria established at the NIH Consensus Development Conference in 1988 [49]. NF1 is caused by heterozygous loss-of-function mutations or deletions of the NF1 gene, encoding the RAS-specific GTPase, neurofibromin, and are observed in the vast majority of affected individuals (> 90%). NF1 is essentially fully penetrant but there is considerable variation in the clinical features of the disease, even within families transmitting the trait. Remarkably, NF1 features might overlap NS in some affected individuals. Such an association, which was named neurofibromatosis-Noonan syndrome (NFNS, OMIM 601321), was first noted more than 25 years ago [50] (Figure 4). It has been long questioned whether NFNS was a variant of NF1 or NS, a chance association, or a distinct disorder [51,52]. Recent reports have provided some insights but have not completely resolved that issue. NF1 mutation analysis performed in three independent cohorts of patients with features fitting NFNS documented occurrence of heterozygous NF1 mutations in 23 of 28 subjects [53-55]. Lesions included nonsense mutations, out-of-frame deletions, missense changes (mostly affecting the GAP domain), and small in-frame deletions. Those groups also excluded

occurrence of PTPN11 mutations in their subjects. In contrast, two cases of NFNS with concomitant NF1 and PTPN11 mutations have been reported [56,57]. NF1 mutations have co-segregated with the NFNS trait in a few kindreds large enough to make second mutations highly unlikely [58]. Taken together, the current evidence indicates that most individuals with NFNS harbor an NF1 mutation and that a single mutation appears to be sufficient to engender the trait. These findings support the view that NFNS is genetically distinct from NS and emphasize the extreme phenotypic variability associated with lesions in the NF1 gene. While the observation of a peculiar NF1 mutational spectrum in NFNS and the fact that these alleles cosegregate in families with the condition suggest that the term “NFNS” primarily characterizes a phenotypic variant of NF1, it should be noted that some of the mutations identified in patients with NFNS have also been reported in NF1 without any feature suggestive of NS.

A phenotype related to NF1 is Legius syndrome (OMIM 611431), an autosomal dominant condition characterized by multiple axillary freckling, café-au-lait spots, macrocephaly, and a NS-like facial dysmorphism in some individuals [59,60]. Some patients have learning difficulties and/or hyperactive behavior. Despite the clinical overlap with NF1, affected individuals do not have Lisch nodules, neurofibromas or central nervous system tumors, while lipomas represent a relatively common feature. This trait is caused by loss-of-function mutations of the SPRED1 gene, which encodes for a negative modulator of RAS-MAPK singnaling [59].

Cardiofaciocutaneous syndrome and Costello syndrome—Cardiofaciocutaneous syndrome (CFCS, OMIM 115150) is a rare, sporadic multiple congenital anomalies/mental retardation syndrome characterized by failure to thrive, severe feeding problems, developmental delay, reduced growth, distinctive dysmorphic facial features, abnormalities of the skin, gastrointestinal tract and central nervous system, and cardiac defects [61,62]. CFCS has considerable clinical overlap with NS, and “borderline” cases are commonly observed, which justified the long debated question of whether it was a separate nosologic entity or an extreme variant of NS. Recurrent craniofacial features include relative/absolute macrocephaly, which is usually associated with high forehead, bitemporal narrowing and facial dysmorphia that is coarser compared to NS (Figure 5). The ectodermal involvement includes dry and hyperkeratotic skin (face, arms and legs), ichthyosis, eczema, sparse, friable and curly hair, and absent/sparse eyebrows and eyelashes. Pigmentary changes (such as café-au-lait spots, nevi or lentigines) and hemangiomata are observed. Heart defects occur in the majority of affected individuals and consist of pulmonic stenosis most commonly, HCM and septal defects. Cognitive deficits are usually moderate to severe. Hypotonia results in marked motor delay. Seizures are also frequently observed. CFCS is genetically heterogeneous, with mutations in the KRAS, BRAF, MEK1 and MEK2 genes occurring in approximately 60-90% of affected individuals [63,64].

Costello syndrome (CS; OMIM 218040) is the eponymous name for the disorder originally described in 1971, and further delineated in 1977, as a condition characterized by prenatal overgrowth followed by severe failure to thrive, distinctive coarse facial features (Figure 5), mental retardation, short stature, cardiac defects (most commonly HCM, septal defects, valve thickening and/or dysplasia, and arrhythmias), and musculoskeletal and skin abnormalities [65,66]. Children and young adults with CS are predisposed to certain malignancies (rhabdomyosarcoma, most commonly), which have been estimated to occur in approximately 15% of affected individuals [67]. Benign cutaneous papillomata in the perinasal or/and perianal region(s) represent a distinctive and common feature associated with the disorder. CS is caused by germline missense mutations in the HRAS gene (OMIM 190020) [68]. The absence of a mutation in the gene in a subject with phenotype apparently fitting CS would be predictive of a clinically related but nosologically distinct condition.

Noonan syndrome and related phenotypes: Genetics and molecular pathogenesis

NS is a disorder caused by aberrant signal flux through RAS and the mitogen activated protein kinase (MAPK) signaling cascade (Figure 6). RAS proteins are small guanosine triphosphate (GTP)/guanosine diphosphate (GDP)-binding GTPases that function as molecular switches controlling a major intracellular signaling network that, depending on the cellular context, guides diverse biological functions such as proliferation, migration, survival, cell fate determination, differentiation and senescence [69,70]. Within this network, signal flow through the RAF-MEK-ERK protein kinase pathway (Figure 6), controls early and late developmental processes, including determination of morphology, organogenesis, synaptic plasticity and growth. Signal transmission via this cascade is initiated by the activation of cell surface receptors by growth factors, hormones and cytokines, which creates intracellular docking sites for adaptor molecules and signal-relay proteins recruiting and activating guanine nucleotide-exchange factors (GEFs). In turn, GEF proteins promote the release of GDP from RAS, favoring binding to the more prevalent GTP and activation of the GTPase. Activated GTP-bound RAS interacts with the RAF serine/threonine kinases (RAF1, BRAF and ARAF) favoring their catalytic activation, which results in the phosphorylation and activation of their substrates, the MAPK/ERK kinases (MEK1 and MEK2). Upon activation, MEKs act as dual specificity kinases to phosphorylate regulatory residues of the extracellular signal-regulated kinases (ERK1 and ERK2), which function as serine/threonine proteine kinases to modulate the activity of a large number of cytoplasmic and nuclear substrates, including proteins implicated in the control of gene expression. Several feedback mechanisms negatively control signal flow through the RAS-MAPK pathway, including the functional inactivation of RAS by GTPase activating proteins (GAPs), which stimulate their intrinsically low GTPase activity. In NS and related diseases, RAS signaling dysregulation can be caused by germline mutations in RAS genes as well as in genes encoding modulators of receptor (CBL) or RAS (PTPN11, SOS1, SHOC2, NF1 and SPRED1) function, or downstream signal transducers (RAF1, BRAF, MEK1 and MEK2). An updated review on disease genes and pathogenetic mechanisms underlying NS and closely related disorders, and their genotype-phenotype correlations follows.

PTPN11—PTPN11 was discovered as a major NS disease gene using a positional candidacy approach ten years ago [71]. The PTPN11 gene (OMIM 176876) encodes SHP2, a cytoplasmic protein tyrosine phosphatase that positively modulate RAS signaling [72]. SHP2 is characterized by two tandemly arranged N-terminal src-homology 2 domains (N-SH2 and C-SH2), a single catalytic domain (PTP) and a C-terminal tail of uncharacterized function. SHP2 binds to phosphotyrosyl-containing signaling partners through the SH2 domains, which control both SHP2's subcellular localization and function [73]. PTPN11 mutations account for approximately 50% of NS, and are more prevalent among subjects with PS and short stature, and less common in individuals with HCM and/or severe cognitive deficits [28,74]. These mutations are almost always missense changes and perturb SHP2's function through distinct mechanisms [75-77]. Most mutations affect residues involved in the N-SH2/PTP interdomain binding network that stabilizes SHP2 in its catalytically inactive conformation, and up-regulate SHP2's function by impairing the switch between the active and inactive conformations, favoring a shift in the equilibrium toward the former. There are, however, changes affecting residues located in the phosphopeptide binding pocket of the N-SH2 and C-SH2 domains or in the linker stretch connecting these domains. These substitutions promote SHP2 gain of function by increasing binding affinity, altering the binding specificity for the signaling partners, or altering the flexibility of the NSH2 domain in a manner that inhibits the N-SH2/PTP interaction.

A distinct class of PTPN11 mutations has been identified to underlie LS [32,33]. Tyr279Cys and Thr468Met represent the most common defects, although additional mutations have

been documented. An impaired catalytic activity has been established as the biochemical behavior shared by these SHP2 mutants [76,78,79]. Their role in SHP2 functional dysregulation still requires further study, since they do not appear to perturb intracellular signaling by a merely dominant negative effect [77,80-82], as supposed in the past.

Children with NS are predisposed to JMML [23]. These children are heterozygous for *PTPN11* germline mutations in the majority of cases, with one mutation rarely observed in NS (218C>T, Thr73Ile) occuring in a large percentage of cases [24]. Remarkably, a distinct class of mutations in this gene, acquired as a somatic event, occurs in approximately one-third of children with non-syndromic JMML and in variable proportions of other myeloid and lymphoid malignancies of childhood [25,83-85]. As observed in NS, the vast majority of *PTPN11* lesions identified in this heterogeneous group of hematologic malignancies are missense changes that alter residues located at the interface between the N-SH2 and PTP domains, but appear to be more activating [86,87].

SOS1—SOS1 (OMIM 182530) is the second most frequently mutated NS disease gene, accounting for approximately 10% of subjects [88-90]. SOS1 encodes a large, multidomain GEF that catalyzes the release of GDP from RAS, facilitating the conversion of the inactive GDP-bound form of RAS to its active GTP-bound state [91]. The vast majority of the mutations identified are missense changes and affect multiple domains. Approximately half of the SOS1 defects affect residues located in the short helical linker connecting the plekstrin homology (PH) and the RAS exchanger motif (REM) domains, with substitutions of residue Arg552 accounting for approximately 30% of total mutations. A second mutation cluster is located within the PH domain (residues 432 to 434), while a third functional cluster resides at the interacting regions of the Dbl homology (Thr266 and Met269) and REM (Trp729 and Ile733) domains. A single amino acid change (Glu846Lys) within the Cdc25 domain accounts for more than 10% of defects. Most of these affected regions are predicted to contribute structurally to the maintenance of the catalytically autoinhibited conformation. Biochemical data confirmed such predictions, demonstrating that NS-causing SOS mutations promote gain of function and enhance RAS and ERK activation [88,89]. Subjects heterozygous for a mutated SOS allele tend to exhibit a distinctive phenotype characterized by ectodermal abnormalities generally associated with a lower prevalence of cognitive deficits and short stature [88,90]. A few SOS mutation-positive individuals with ectodermal manifestations and distinctive facial dysmorphia that might be suggestive of CFCS have been reported. In these subjects, however, cognitive deficits are generally absent or minor.

KRAS and NRAS—KRAS (OMIM 190070), NRAS (OMIM 164790), and the structurally and functionally related HRAS (OMIM 190020) are monomeric GTPases that use GDP/GTP-regulated molecular switching to control intracellular signal flow [69,70]. They exhibit high affinity and binding specificity for GDP and GTP, low GTPase activity, and cycle from a GDP-bound inactive state to a GTP-bound active state, the latter favoring their interaction with multiple downstream transducers. These proteins are activated by GEFs, including SOS1, which promote release of GDP, and inactivated by GAPs, which accelerate the intrinsic GTPase activity. Heterozygous germline mutations in each of the three members of the RAS subfamily have been identified to be causally related with NS (KRAS and NRAS), CFCS (KRAS) or CS (HRAS) [63,64,68,92,93].

Germline KRAS mutations account for a relatively small percentage of affected subjects in both NS and CFCS (< 2-5%), and, in NS, are generally associated with a highly variable but generally severe phenotype. The diversity of mutations associated with these developmental disorders as well as their phenotypic spectrum have been investigated further, refining the picture of a clustered distribution of germline disease-associated KRAS defects, and

confirming the high clinical variability [94,95]. As observed for the somatically acquired oncogenic RAS gene mutations and germline transmitted HRAS lesions, the NS/CFCS-causing KRAS defects up-regulate protein function by either affecting the intrinsic and/or GAP-stimulated GTPase activity and, consequently, impairing the switch between the active and inactive conformation, or determining a dramatically increased rate of guanine nucleotide dissociation [26,96].

More recently, two germline missense mutations of NRAS (Thr50Ile and Gly60Glu amino acid changes) have been reported to account for a few NS cases [93]. Functional characterization of these disease-associated mutations documented that, similarly to what was observed for KRAS and HRAS mutation, they confer enhanced stimulus-dependent MAPK activation but apparently through different molecular mechanisms.

RAF1 and BRAF—RAF1 (OMIM 164760), BRAF (OMIM 164757) and ARAF (OMIM 311010) are members of a small family of serine-threonine kinases that function as RAS effectors [97,98]. These kinases phosphorylate and activate the dual specificity kinases MEK1 and MEK2, which in turn promote the activation of the MAPKs, ERK1 and ERK2. The three members of the RAF family play different roles in the activation of the RAS-MAPK signaling cascade. BRAF has considerably higher MEK kinase activity compared to RAF1 and ARAF. These proteins also differ in their expression profiles as well as in the regulatory mechanisms controlling their function, and appear to have unique roles during development. Consistent with these observations, somatic BRAF mutations frequently occur in malignant melanomas and in thyroid, colorectal and ovarian cancers, while ARAF and RAF1 missense changes are observed rarely in malignancies (COSMIC database, http://www.sanger.ac.uk/genetics/CGP/cosmic/).

Two independent surveys identified missense mutations in RAF1 in subjects with NS with a prevalence of mutations ranging between 5-15% [34,99]. Mutations in the same gene were also identified in two of six subjects with LS without a mutation in PTPN11 [34]. RAF1 mutations affect residues clustering in three regions of the protein. The first cluster (70% of total RAF1 defects) involves the N-terminal consensus 14-3-3 recognition sequence or adjacent residues. The second group (15% of RAF1 lesions) includes mutations affecting residues within the activation segment region of the kinase domain (Asp486 and Thr491). Of note, several *BRAF* missense mutations detected in solid tumors alter this amino acid stretch, including some (Asp594Gly and Thr599Ile) homologous to those of RAF1 identified in subjects with NS. The third cluster (15% of RAF1 mutations) affect two adjacent residues (Ser612 and Leu613) located at the C-terminus. Functional characterization of a selected panel of RAF1 mutants supports the idea that mutations can differentially perturb protein function and intracellular signaling. In particular, mutations affecting the N-terminal 14-3-3 binding motif or the C-terminus of the protein, promote enhanced kinase activity and increased activation of the MAPK cascade compared to wild-type protein [34,99]. In contrast, Asp486Asn and Thr491Ile, representing the mutation cluster in the activation segment, were observed to be kinase impaired and differentially perturb signaling through the MAPK cascade. Pandit and coworkers [34] provided evidence supporting the model that the increased activation promoted by the amino acid substitutions affecting the N-terminal 14-3-3 binding site results from a loss of 14-3-3-mediated inactivation, required to stabilize the RAF1's inactive state by favoring the interaction of the N-terminal portion of the protein with the kinase domain at the C-terminus. Phenotype analysis of the NS and LS subjects with RAF1 mutations is notable for the observation that a large percentage of cases (75%) exhibit HCM, compared to HCM prevalence of 18% in the general NS population [34,99]. This genotype-phenotype correlation seems to be allele-specific. In addition to the two cases with LS, multiple nevi, lentigines and/or café-au-lait

spots were detectable in one-third of NS patients with RAF1 mutations, suggesting a predisposition to hyperpigmented cutaneous lesions.

Germline BRAF mutations have been documented in a small percentage of subjects with phenotypes fitting or suggestive of NS or LS (< 2% of cases) [35,36,99,100]. Of note, BRAF had previously beed identified as a major disease gene underlying CFCS (50-75% of cases) [63,64]. NS-associated mutations largely do not overlap with those occurring in CFCS, suggesting a genotype-phenotype correlation. Among subjects with NS, the available clinical data indicate an associatein with neonatal growth failure and feeding difficulties, mild-to-moderate cognitive deficits, and hypotonia, and a higher prevalence of multiple nevi and dark colored lentigines [35]. As adults, they display a phenotype that is more severe compared to those associated with PTPN11 and SOS1 mutations. In these individuals, however, polyhydramnios, HCM, and CFCS-related skin features are uncommon or absent, and cardiac defects, neurological impairment and feeding problems appear to be less severe compared to what is generally observed in CFCS [35]. Of note, while the occurrence of distinct BRAF gene mutations in subjects with a phenotype fitting NS would indicate that some features might be allele-specific, the observation that a subgroup of BRAF mutation-positive subjects exhibits an “intermediate” phenotype suggests that a clinical continuum characterized by a differential combination and severity of features is associated with defects in the BRAF gene. The observation of a patient with LS and normal intelligence who was found to carry a novel sequence change in BRAF [36] further illustrates that the phenotypic spectrum caused by BRAF mutations is broader than previously assumed and that mental retardation is not an invariably associated feature. To date, more than 40 germline BRAF mutations have been identified. All are missense defects that cluster in the cysteine-rich domain and in the amino-terminal portion and activation segment of the kinase domain. Most mutations are recurrent, with substitutions of residues Gln257 and Glu501 accounting for approximately 40% of all defects.

Germline BRAF mutations are rarely observed as somatic mutations contributing to oncogenesis and, as observed for the oncogenic lesions, they can confer either increased or decreased activity upon the mutant protein [35,64], suggesting that multiple alternative mechanisms are likely to be involved in the functional dysregulation of the kinase.

SHOC2—The invariant 4A>G missense (Ser2Gly) change in the SHOC2 gene (OMIM 602775), which encodes a protein that positively modulates RAS-MAPK signal flow [94-96], was recently discovered to underlie NS/LAH [101-103]. This amino acid change was demonstrated to promote N-myristoylation of the protein, and causes aberrant targeting of SHOC2 to the plasma membrane and impaired translocation to the nucleus upon growth factor stimulation. SHOC2 is a widely expressed protein composed almost entirely by leucine-rich repeats. SHOC2 is believed to positively modulates RAS-MAPK signaling by promoting protein phosphatase 1C (PP1C)-mediated RAF1 dephosphorylation at residue Ser259, which is required for stable RAF1 translocation to the plasma membrane and catalytic activation [103]. Initial functional characterization of the mutant protein indicates that the expression of $SHOC2^{S2G}$ mutant enhances ERK activation in a cell type-specific fashion [42]. The observations that subcellular localization of SHOC2 is restricted to the nucleus following EGF stimulation and that $SHOC2^{S2G}$ ectopic expression in *C. elegans* engenders a neomorphic phenotype previously associated with aberrant signaling, however, suggest that the disease-causing mutant might exert a wider perturbing effect on intracellular signaling.

MEK1—MEK1 (OMIM 176872), and the functionally related MEK2 (OMIM 601263), belong to a family of dual specificity kinases that phosphorylate ERK proteins at tyrosine and serine/threonine residues [104]. MEK1 and MEK2 are both effectors of RAF proteins,

but appear to play non-redundant roles. Missense mutations and in-frame deletions in MEK1 and MEK2 account for approximately 20% of CFCS [64,105-108], but one germline missense mutation in MEK1, predicting the Asp67Asn amino acid substitution, has been documented in two unrelated subjects exhibiting a phenotype fitting NS [108]. CFCS-causing MEK gene mutations affect residues located in the regulatory region of the protein and promote gain of function of the kinase [64], while no datum on the effect of the Asp67Asn change on signaling is currently available.

CBL—Martinelli and co-workers recently reported that heterozygous germline mutations in CBL can underlie a phenotype with clinical features fitting or partially overlapping NS [44]. Independent CBL mutations were identified in two sporadic cases and two families from among 365 unrelated subjects with NS (or suggestive features) and negative for mutations in previously identified disease genes. Phenotypic heterogeneity and variable expressivity were documented. Consistent with this finding, two unrelated surveys documented developmental anomalies reminiscent of NS in subjects heterozygous for a germline CBL mutation [45,46]. CBL is a member of a small family of E3 ubiquitin ligases that negatively regulate intracellular signaling downstream of receptor tyrosine kinases (RTKs), but also contribute to signal traffic by its adaptor function [109]. CBL mediates the conjugation of ubiquitin to activated RTKs, which is required for receptor internalization, endocytic sorting, and switching off signaling via receptor degradation or recycling [110]. Somatically acquired CBL mutations occur with variable prevalence in myeloid malignancies, including JMML, and are generally observed as homozygous lesions due to acquired isodisomy [111]. Leukemia-associated mutations are largely small in-frame deletions, splice site or missense changes affecting the RING finger domain and/or the adjacent linker region, and appear to act in a dominant-negative fashion by uncoupling CBL binding to activated RTKs from their ubiquitylation and degradation. Of note, the spectrum of germline CBL mutations overlaps that of leukemia-associated lesions. Functional characterization of a panel of mutants documented that mutations affect CBL-mediated receptor ubiquitylation and dysregulate signal flow through RAS [44,46]. These recent findings provide evidence that CBL functional dysregulation can significantly perturb a wider range of cellular processes than previously known, with direct impact on development.

Summary

Recent discoveries derived from a massive disease gene hunting effort have established that NS, one of the most common developmental disorders in man, is caused by genetic lesions promoting upregulation of RAS signaling. These discoveries also documented that aberrant activation of RAS signaling, particularly through the MAPK cascade, underlies a number of clinically related disorders that today are grouped within the NCFCS family (also known as RAS-opathies). Based on the relatively high prevalence of some of these disorders, the dysregulation of this signaling pathway also represents one of the most common events affecting developmental processes.

In NS, screening efforts and genotype-phenotype correlation studies have documented that mutations in the identified disease genes are associated with distinct phenotypes, explaining, in part, the wide clinical diversity characterizing this Mendelian trait. The overlapping features observed among these related disorders, the wide breadth of phenotypes within each trait, and absence of clinical features with pathognomonic value and consensus on specific and routinely used diagnostic criteria make diagnosis of NS and related diseases challenging. This has direct impact on patient management because of the prognostic relevance of diagnosis. Molecular diagnosis now offers the opportunity to overcome the weakness of subjective clinical criteria, and represents a highly informative prognostic tool, with direct impact on counseling and appropriate patient management.

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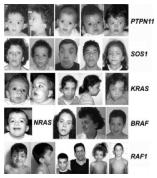

Figure 1. Facial dysmorphism in Noonan syndrome

Series of affected individuals heterozygous for mutations in different disease genes are shown. (Kindly provided by G. Zampino, M.C. Digilio, B. Dallapiccola and G.B. Ferrero)

Figure 2. Facial dysmorphism in LEOPARD syndrome

Series of affected individuals heterozygous for mutations in PTPN11 are shown. (Kindly provided by M.C. Digilio and B. Dallapiccola)

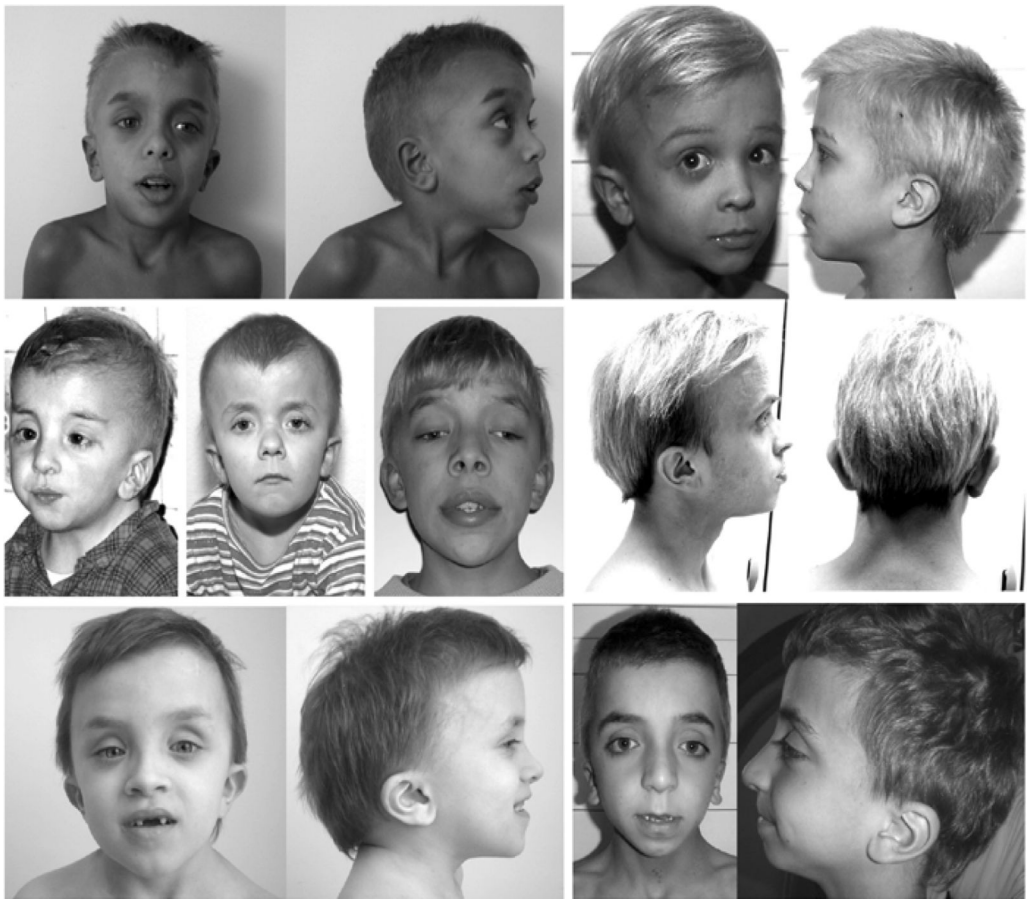

Figure 3. Facial dysmorphism in Noonan-like syndrome with loose anagen hair

Series of affected individuals heterozygous for the 4A>G missense change (Ser2Gly) in SHOC2 are shown. (Kindly provided by G. Zampino, M.C. Digilio and B. Dallapiccola)

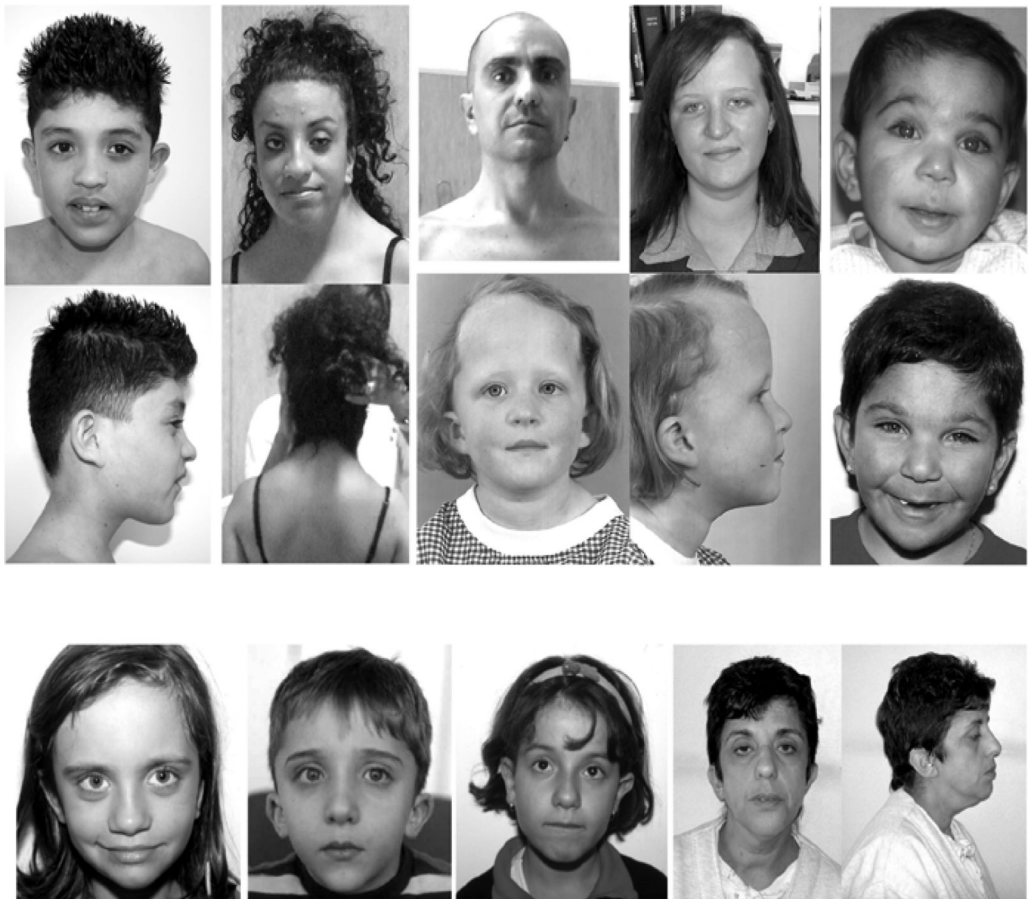

Figure 4. Facial dysmorphism in CBL mutation-associated syndrome and neurobibromatosis-Noonan syndrome

Series of affected individuals heterozygous for germline CBL (above) or NF1 (below) mutations are shown. (Kindly provided by G. Zampino, M.C. Digilio, B. Dallapiccola, M.L. Cavaliere, J.M. van Hagen and R. Savarirayan)

Figure 5. Facial dysmorphism in cardiofaciocutaneous syndrome and Costello syndrome

Series of affected individuals with cardiofaciocutaneous syndrome (above and middle panels) and Costello syndrome (below panel) are shown. (Kindly provided by G. Zampino, M.C. Digilio, B. Dallapiccola and G. Mancini)

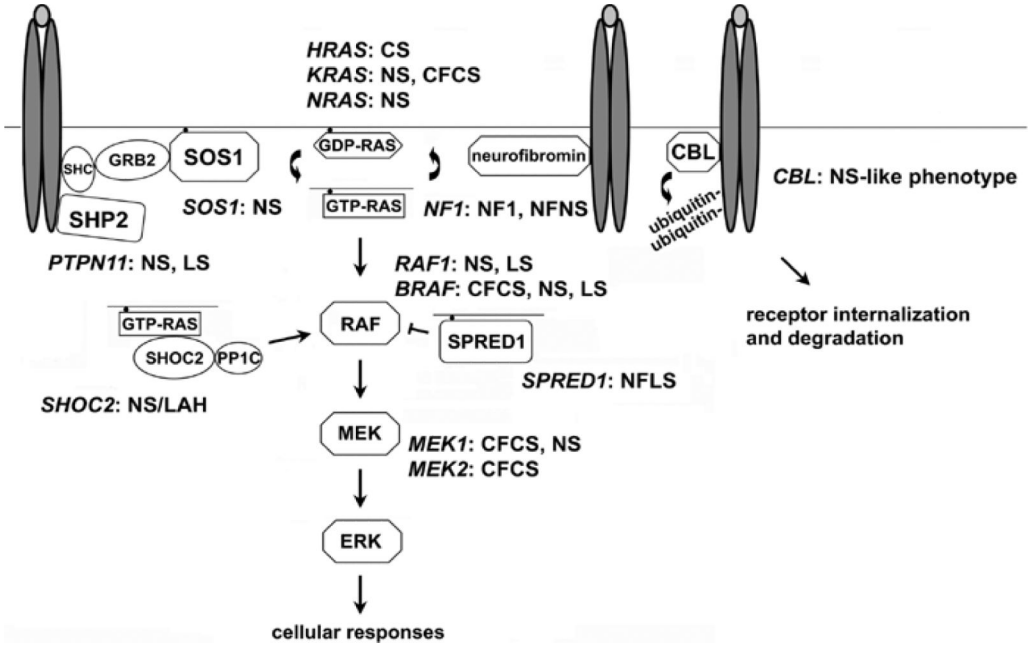

Figure 6. The RAS-MAPK signal transduction pathway

Schematic diagram showing the RAS-MAPK cascade and affected disease genes in disorders of the neuro-cardio-facial-cutaneous syndrome family. The double ovals in dark grey and the light grey ovals represent generic dimerized cell-surface receptors binding to their ligand. Abbreviations: CFCS, cardiofaciocutaneous syndrome; CS: Costello syndrome; LS, LEOPARD syndrome; NF1, neurofibromatosis type 1; NFLS, Neurofibromatosis type 1-like syndrome (also termed Legius syndrome); NFNS, neurofibromatosis-Noonan syndrome; NS, Noonan syndrome; NS/LAH, Noonan-like syndrome with loose anagen hair.

Table 1

Clinical Features of Noonan Syndrome

Facial dysmorphism
Epicanthal folds
Ptosis
Down-slanting palpebral fissures
Triangular facies
Low set and/or posteriorly rotated ears
Light colored irises
Ophthalmologic
Strabismus
Myopia
Hearing Loss
Dental/Oral
Malocclusion
High arched palate
Cardiovascular
Congenital heart defects
Pulmonic stenosis
Atrioventricular septal defects
Aortic coarctation
Secundum atrial septal defects
Mitral valve defects
Tetralogy of Fallot
Ventricular septal defects
Patent ductus arteriosus
Hypertrophic cardiomyopathy
Webbed neck with low posterior hairline
Feeding difficulties
Postnatally reduced growth
Developmental
Delay
Attention deficit/hyperactivity disorder
Skeletal
Pectus excavatum and/or carinatum
Cubitus valgus
Scoliosis
Vertebral anomalies
Cryptorchidism
Lymphatic
Lymphedema
Lymphangiectasia

Hematological
Bleeding diathesis
Thrombocytopenia
Leukemia