Noonan syndrome and Rasopathies: clinical features, diagnosis and management

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Abstract

Noonan syndrome (NS) and NS-related disorders (**C**ardio-**F**acio-**C**utaneous (CFC) syndrome, Costello syndrome, LEOPARD (**L**entigines, **E**CG conduction abnormalities, **O**cular hypertelorism, **P**ulmonic stenosis, **A**bnormal genitalia, **R**etardation of growth and sensory neural **D**eafness) syndrome share common clinical features characterized by unique facial features, postnatal growth failure, psychomotor retardation, ectodermal abnormalities, congenital heart diseases, chest & skeletal deformity and delayed puberty. During last decade, strident progress has been made in molecular understanding of NS. The functional alterations of the Ras-mitogen-activated protein kinase (MAPK) pathway are caused by the mutation in more than 10 genes (*PTPN11, SOS1, RAF1, SHOC2, BRAF, KRAS, NRAS, HRAS, MEK1, MEK2*). Therefore, NS and NS-related

disorders are called RASopathies as a disease group. *PTPN11* (40-50%), *SOS1* (10%–20%), and *RAF1* (3%-17%) mutations are common in NS patients. In this review, the constellation of overlapping clinical features of RASopathies will be described based on genotype as well as their differential diagnostic points and management.

Key words: Noonan syndrome, Mutation, Cardiofaciocutaneous syndrome, Costello syndrome, LEOPARD syndrome

Introduction

Noonan syndrome (NS; OMIM 163950) is one of the most common autosomal dominant growth and developmental disorders, which was originally described by Jacquelin Noonan, a pediatric cardiologist in 1962[1)]. An affected parent is present in 14-75% of the affected children. Although the textbook-wise incidence of affected individuals is estimated to be 1/1,000 and 1/2,500, it seems to be not common or under-diagnosed particularly in subtle cases[2)]. In Korea, more than 100 Noonan patients are enrolled in registry. LEOPARD syndrome (LS; OMIM 151100) (**L**entigines, **E**CG conduction abnormalities, **O**cular hypertelorism, **P**ulmonic stenosis, **A**bnormal genitalia, **R**etardation of growth and sensory neural **D**eafness), cardio-facio-cutaneous syndrome (CFC; OMIM 115150), and Costello syndrome (CS; OMIM 218040) exhibit overlapping phenotypes with NS and are categorized as NS-related disorders[3, 4)]. They are all clinically characterized by the constellation of dysmorphic face, congenital heart disease, proportionate post-natal short stature, chest deformity, delayed puberty, short neck, dermatological abnormalities, and hematological abnormalities. During past ten years, a strident progress has been made in the understanding of molecular defects of Noonan syndrome since Tartaglia et al. identified the heterozygous mutation in *PTPN11*(protein tyrosine phosphatase, non-receptor type 11) in 2001[5)]. As of 2012, more than 7 genes (*PTPN11, KRAS, SOS1, RAF1, BRAF, SHOC2, NRAS*) were known to cause Noonan syndrome and its related disorders[6)]. Since the consequences of genetic defect of these genes are the gain of function in the RAS (rat sarcoma viral oncogene/MAPK(mitogen-activated protein kinase) pathway, Noonan syndrome and its related disorders (LEOPARD, CFC, Costello syndromes, and Neurofibromatosis type I)

are re-grouped as RASopathies[7]. Because of these overlapping phenotypic and molecular genetic characteristics among NS and NS-related disorders, the clinical delineation of these syndromes is confusing and problematic. However, accurate diagnosis is critical for proper management as well as genetic counseling.

Genetic heterogeneity and molecular genetics of Noonan syndrome and RASopathies

The molecular genetic pathogenesis of NS and NS-related disorders is related to functional alterations of the Ras-mitogen-activated protein kinase (MAPK) signaling pathway, which is implicated in growth factor-mediated cell proliferation, differentiation, and apoptosis. RAS proteins (HRAS, NRAS and KRAS) are small guanosine binding proteins, playing a role of a signal switch that integrates extracellular stimuli and activates downstream effectors. The GTP-bound form of RAS can activate several intracellular pathways including MAPK pathway. The RAS down-stream effector pathway is RAF (murine sarcoma viral ongogene homolog)-MEK (mitogen-activated kinase kinase)-ERK (mitogen-activated kinase) pathway. There are three RAF serine/threonine kinase (ARAF, BRAF, and RAF1), activating the MEK-ERK kinase cascade. PTPN11 and SOS1 (son of sevenless1) are in the upstream of RAS. Most patients carrying the mutation in these genes are Noonan or LEOPARD syndrome phenotypes. Gain-of-function germ-line mutations affecting components of the Ras-MAPK pathway are involved in the development of NS and NS-related disorders[8]. *PTPN11* (40-50%), *SOS1* (10%–20%), and *RAF1* (3%-17%) mutations are common in NS patients[8-11]. A small number of NS patients can also harbor *KRAS*[12], *BRAF*[13],

MEK1 [4], or *NRAS* mutations[14]. Recently, *SHOC2* mutation was identified in NS-like patients with loose anagen hair[15]. Still, causative mutations are unknown in 30%–40% of patients with NS[8, 11]. A total of 60%–80% of CFC patients harbor *BRAF* (~50%), *MEK1* and *MEK2* mutations[4, 13, 16-19]. *PTPN11*, *RAF1* and *BRAF* mutations have been described in most patients (95%) with LS, and *HRAS* mutations are present in most cases with CS[2, 13, 20, 21]. In Korean patients, *PTPN11* (39.0%) and *SOS1* mutations (20.0%) were the common mutations in NS, as we previously reported[22], whereas *RAF1*, *KRAS*, and *BRAF* mutations were less common. Importantly, the full mutation spectrum of CFC has not been reported. In our study on Korean patients, *BRAF* (41.2%) and *SHOC2* (23.5%) mutations were the most common mutations in CFC. Additional mutations in *MEK1*, *MEK2*, *KRAS*, and *SOS1* can be also identified in a small number of CFC patients[11, 22]. *SHOC2* mutations were previously reported in patients with NS-like features[15]. On the other hand, in our study on Korean patients[22], *SHOC2* mutations were not identified in NS patients but in a substantial proportion of CFC patients as in a recent report by Komatsuzaki et al.[23], indicating that *SHOC2* mutations can be identified in CFC as well as NS and they might be more common in CFC. CS and LS were characterized by a few representative mutations in *HRAS* and *PTPN11*, respectively. Still, there exists phenotypic heterogeneity even in a small number of patients with a same genotype. As described in a *SHOC2* mutation, a *BRAF* mutation, p.G464R, was previously identified in a CFC patient[3], but in a NS patient in our report on Korean patients[22]. These phenotypic overlap can be found in other genes including *MEK1*, *MEK2*, and *KRAS* as well[4]. The functional effects of the three variants identified in our Korean cohort, one novel and two previously described[11], were verified by exploring the downstream effectors in the Ras-MAPK pathway[22]. RAF1 is

activated by GTP-bounded RAS and phosphorylates serine residues of MEK. In this process, the conserved region 2 (CR2) domain of RAF1 plays a major role. Recently, Kobayashi et al. demonstrated that the dephosphorylated status of p.S259 in the CR2 domain is important for the activation of RAF1. Notably, most *RAF1* mutations, including all of those in our cohort, are found in the CR2 domain[11, 20, 22, 24]. In particular, two novel mutations, p.S259T and p.P261T, were located at or near the p.S259 residue. Accordingly, their in vitro activities were higher than those of wild-type RAF1 in the presence of growth factor[22]. On the other hand, p.K170E in SOS1 is located in the HF domain, where NS mutations have rarely been reported. The HF domain is predicted to block the allosteric Ras-binding site and prevents Ras activation by SOS1, stabilizing the autoinhibitory conformation of SOS1 in the resting state. In the presence of a growth stimulus, this blocking is unlocked and Ras binds to SOS1 and is activated[25, 26]. Despite extensive efforts to identify disease-causing genes in NS and NS-related disorders, approximately 30% of patients with NS or CFC remain genetically undiagnosed[4, 8-10], as in our studies[11, 22]. Further study is needed to identify new disease-causing gene(s) in NS with unknown genotypes in order to fully understand the molecular pathophysiology of the disorder.

Genotype-phenotype correlation

Differential diagnoses of NS and NS-related disorders are clinically meaningful because their prognosis and management are different; Short stature and global developmental delay or mental retardation are more pronounced in patients with NS-related disorders than in patients with NS. Characteristic craniofacial and ectodermal findings, such as

sparse hair, sparse eyebrow, ichthyosis, deep palmar crease, and hyperkeratosis, are important clues for the differential diagnosis, but they can be ambiguous as well. Moreover, the differential diagnosis is problematic in very young patients like infant and toddlers. Identification of more specific correlations between genotypes and phenotypes emphasizes the importance of genotype-based surveillance and management. For instances, pulmonary stenosis, pectus deformity easy bruising and hematological malignancies are associated with *PTPN11* mutations[5, 27], whereas hypertrophic cardiomyopathy shows a strong association with *RAF1* mutations[11, 20]. In our study on Korean patients, pulmonary stenosis was also associated with *SOS1* mutation (83.3%), as recently reported by Kobayashi (~70%)[22, 28]. In addition, solid tumor surveillance is important in patients with *HRAS* mutations[21]. A low prevalence (41.7%) of short stature was observed in Korean patients with *SOS1* mutation as in previous reports[22, 25, 29]. NS patients carrying *SOS1* mutation are more likely to CFC syndrome like skin findings and less likely to have short stature and impaired cognitive function. SOS1 may not be directly related to the JAK-STAT (Janus kinase-signal transducers and activators of transcription) pathway, a major signaling pathway induced by growth hormone (GH) that is directly linked to PTPN11 and negatively regulated by it[30]. NS patients with *SHOC2* gene mutation tend to have higher frequency of mitral valve prolapse and septal defects. They are more likely to have growth hormone deficiency. Also, they have easily pluckable, sparse, thin, slow growing hair, darkly pigmented skin eczema, and icthyosis. They frequently present with hypernasal voice. Considering the high prevalence of short stature in NS-related disorders, the relationships between Ras-MAPK proteins besides PTPN11 and the signaling pathways induced by GH should also be evaluated. In addition, the high prevalence of global developmental delay and mental retardation in

patients with *HRAS*, *BRAF*, *KRAS*, and *SHOC2* mutations compared to those with *PTPN11* and *SOS1* mutations suggests that mental retardation might be influenced by differences in the nature of disease-causing genes in the Ras-MAPK pathway[6].

Clinical features, differential diagnosis and management

1. Noonan syndrome

Traditionally, van der Burgt's criteria[1] have been utilized to make NS diagnosis. The typical face with one or two major clinical characteristics or suggestive face with two major or three minor clinical characteristics is required to reach the diagnosis. Short stature (postnatal onset) is usually observed in 50-80% of patients. Birth weight and height are typically normal, but there is a substantial deceleration of growth velocity during 2-4 years after birth with more than 2 years delayed bone age for chronological age. Final adult height of NS reaches the lower limit of normal at the end of the second decade of life, 160-162 cm in males, 150-152 cm in females in non-Asians. Head and neck abnormalities are often striking including triangular face, ear abnormalities (44-90%) with low-set posteriorly rotated ears with thick helix, sensory neural hearing loss, eye abnormalities (95%) with ptosis, hypertelorism, down-slanting palpebral fissures, strabismus, proptosis, myopia and nystagmus, deeply grooved philtrum with high peaks of upper lip vermillion border (95%), neck abnormalities (95%) with short or webbed neck, high arched palate (34-45%), dental malocclusion (35%), low posterior hair line (32%), and micrognathia (22%). Congenital heart defects are frequently accompanied in 50-75% of patients, most commonly pulmonic valve stenosis (50%). Other cardiovascular abnormalities are hypertrophic cardiomyopathy (20 - 30%), atrial septal

defect (10%), and others (aortic stenosis, ventricular septal defect and mitral insufficiency). Those with cardiac problems should have regular follow-up at intervals determined by the cardiologist. Some will require treatment such as balloon valvuloplasty or surgery. Long-term reevaluation of these patients after treatment is essential (specifically, after successful cardiac surgery, cardiac care should not be discontinued). Individuals without heart disease on their initial evaluation should have cardiac reevaluation every 5 years. Adults should not discontinue periodic cardiac evaluations even if their evaluations in childhood or adolescence were normal because unexpected cardiac findings can occur at any point in time[6]. Chest deformities are one of the major criteria for the diagnosis, demonstrated in 53-70% of patients; flat, funnel, shield or deformed chest, pectus carinatum superiorly and or pectus excavatum inferiorly. Cryptoorchidism in males and delayed puberty are common problems in 60-80% of patients. The mean age of pubertal onset is 13.5-14.5 years in boys and 13-14 years in girls. Skeletal abnormalities are cubitus valgus (47%), hand abnormalities including clinodactyly, brachydactyly and blunt finger tips (30%), and vertebral abnormalities (25%). Neurological involvements are featured by motor developmental delay (26%), language delay (20%), learning disability (15%), recurrent seizure in 13% peripheral neuropathy in 3% and mild mental retardation (25-35%). Hematological problems are noted in NS patients; bleeding diathesis (20%) including factor XI or XII deficiencies, von Willebrand's disease, platelet dysfunction and leukemia, especially juvenile myelomonocytic leukemia (JMML)[31].

The diagnosis of NS is primarily based on clinical features aforementioned. Therefore, high clinical suspicion should be a prerequisite for the diagnosis. Mutation analysis of six genes (*PTPN11*, *SOS1*, *RAF1*, *SHOC2*, *KRAS*, *NRAS*) makes the diagnosis

confirmatory in about 60-70%. In female patients, karyotype should be done to exclude Turner syndrome[6, 22, 31].

Management of clinical problems encountered in NS patients is entirely dependent on its specific manifestation. Careful physical examinations are mandatory to delineate the presence of congenital defects in cardiovascular, genitourinary, skeletal, and other birth defects. Documentation of past growth history is helpful to understand growth problem. Audiometric and ophthalmologic evaluations are needed. Surgical intervention is required for congenital heart defects and cryptoorchidism. A mutation in *RAF1* gene is frequently associated with hypertrophic cardiomyopathy observed in 20% of NS patients. Before the surgery, bleeding diathesis should be ruled out by testing bleeding time, coagulation profiles and peripheral blood smear. Also the risk for malignant hyperthermia has to be considered in choosing anesthetics. Mild myeloproliferative disorder in 10% of NS infants, resembling juvenile myelomonocytic leukemia is usually improved by 1 year of age without specific therapy. The incidence of cancer in NS adults is 3.5 fold higher than in general population, commonly hematological malignancies and neuroblastoma[32]. Special education might be required in 10-40% of NS patients. However, NS patients carrying the mutation in the *SOS1* gene and N380D or N380S mutation in the *PTPN11* gene do not show cognitive impairment. Most NS infants have feeding difficulties with poor suck and prolonged feeding time and may require tube feeding for longer than 2 weeks in 24% of NS infants. The period of failure to thrive is usually self-limited within first 2 years after birth. Oral and dental problems also frequently draw an attention. Particularly, dental malocclusion are commonly observed (50-67%), necessitating orthodontics intervention. Articulation difficulty is common (72%), but responds well to intervention therapy. Language delay may be

originated from hearing loss, perceptual motor disabilities or articulation deficiencies[2, 6]. Growth evaluation is important in individuals with postnatal growth failure or delayed puberty. IGF-1 and IGF-BP3 levels are measured with thyroid function test. Most NS patients show normal levels of IGF-1 and IGF-BP3, indicating growth hormone (GH) deficiency is not culpable for postnatal growth failure. However, some studies have demonstrated subnormal overnight mean growth hormone concentration, suggestive of impaired GH secretion. There are several reports from a recombinant human growth hormone (rhGH) registry, the KIGS International Growth Database[33, 34], as well as several, observational studies reporting the effects of rhGH on the near-adult height of subjects with NS[35-37]. Although these studies involved small numbers of patients with various enrollment ages, treatment durations, rhGH doses, and treatment response, rhGH therapy for NS has been reported to be effective to improve both the height velocity and the final adult height. The National Cooperative Growth Study (NCGS), a post-marketing observational study of rhGH-treated children, showed that rhGH significantly improved height SDS in relatively large numbers of children with NS[38]. In May 2007, NovoNordisk obtained FDA approval for the treatment of NS with their rhGH preparation, norditropin using a dose of 66 μg/kg/day[39]. It has been suggested that there may be a genotype-phenotype correlation with respect to spontaneous growth, IGF-1 & IGF-BP3 levels, and response to GH therapy[5, 25]. NS patients with PTPN11 mutation tend to be born with shorter birth length, lower IGF-1, IGF-BP3, higher resting and stimulated GH levels, and poorer response to GH therapy. These phenomena hypothetically have been explained by post-receptor signaling defect. The frequently detected upstream defects of this pathway are gain-of-function mutations of *PTPN11*, which are associated with a mild form of GH resistance and IGF-1 deficiency,

presumably due to interference with the Janus kinase 2, and the signal transducer and activator of transcription 5b (JAK2-STAT) signaling of the GH receptor. In approximately half of all individuals with NS, the cytoplasmic tyrosine phosphatase SHP2 encoded by *PTPN11* is mutated[31]. However, many conflicting reports were published. The long-term data regarding adult heights for those treated with GH showed no difference between mutation-positive and negative patients. Several studies have reported final height data after rhGH treatment of children with NS[33-37]. However, these studies were relatively small and they lacked the matched or randomized controls required for proper comparison. The height gain varied largely in these studies (0.6–1.8 SDS, mean height gain: 9.5-13 cm for boys and 9.0-9.8 cm for girls), with the best results reported in those performed in younger age groups at the start of treatment. Interestingly, either the younger age the rhGH treatment is started or the older age of the start of puberty, the better the result[35-37]. Recently, our group published a result of efficacy of GH therapy in PTPN11 mutation positive or negative Korean Noonan patients. This study showed the efficacy of short-term growth promotion in both groups[40]. Other endocrine issues are delayed puberty and autoimmune thyroid disorder. Low dose estrogen or androgen for pubertal induction may be instituted for girls at age of 13 years or more and boys at age of 14 years or more without secondary sexual characteristics[2].

2. LEOPARD syndrome

Acronym LEOPARD denotes the **L**entigines, **E**KG abnormalities, **O**cular hypertelorism, **P**ulmonic stenosis, **A**bnormalities of genitalia, **R**etardation of growth, and **D**eafness. The syndrome mimics NS clinical features; hypertelorism, pumonic

valve stenosis and hypertrophic cardiomyopathy, and short stature. More striking features of LEOPARD syndrome are multiple skin lentigines, especially on neck and trunk, sensory neural hearing loss, conduction abnormalities on EKG(prolonged PR, QRS intervals and abnormal P waves). They tend to be more mentally retarded. Delayed puberty and hypogonadotrophic hypogonadism may require sex hormone therapy. Less commonly, there are cleft palate, renal agenesis, and kyphoscoliosis. From the molecular point of view, LEOPARD syndrome is allelic with NS in the *PTPN11* gene, more than 90% LEOPARD patients carry Y259C and T468M, which are not found in NS patients [2, 6, 22, 31].

3. CFC syndrome

Cardio-facio-cutaneous syndrome mimics many clinical features with NS; they include hypertelorism with down-slanting palpebral fissures, epicanthic folds, and eyelid ptosis, depressed nasal root, short stature, relative macrocephaly, pulmonic valve stenosis, hypertrophic cardiomyopathy, and ASD. However, they present with relatively coarse face with high forehead and bitemporal depression, failure to thrive and constipation, and moderate to severe psychomotor delay. Most unique features are present in ectoderm, consisting of dry, hyperkeratotic, scaly skin, sparse and curly hair, absent or sparse eye brows and lashes, and keraratosis pilaris. The diagnosis is based on typical clinical features including ectodermal abnormalities. DNA testing provides a confirmatory diagnosis in 70% of patients; two thirds of patients carry a mutation in *BRAF* gene, and rest of them in *MEK1* and *MEK2* genes. Prenatally, pregnancies are often associated with polyhydramnios. Prematurity is common. Prenatal ultrasonogram may demonstrate the fetus with macrosomia, ventriculomegaly, increased nuchal fold

thickness, or evident hydrops fetalis. Specific management should be based on each clinical problem. Feeding and GI problems are common, requiring nasogastric tube feeding in infancy. Short stature is found in most patients (>70%), where GH therapy is not justified at the present. Congenital heart diseases requiring surgical intervention are found in 75% of patients; pulmonic valve stenosis is most common (45%), followed by atrial septal defect(30%), ventricular septal defect(22%). Hypertrophic cardiomyopathy is frequently accompanied in 40% of patients, sometimes necessitating the medication of β-blocker or calcium channel blockers. Nutritional education is necessary to avoid chronic constipation. Topical emollient and keratolytic preparations might be used for the amelioration of skin problems. Opthalmologic evaluation should be performed periodically. Myopic, optic nerve hypotrophy, and nystagmus are common. Cryptoorchidism and renal/bladder abnormalities are present in 20-38%. Genital evaluation and renal sonogram should be done at the diagnosis. Musculoskeletal system is also evaluated since kyphoscoliosis and joint contractures are common[6, 22, 31, 41].

4. Costello syndrome

Costello syndrome is a unique congenital anomaly syndrome caused by a gain of function mutation in the oncogene, *HRAS* gene with increased risk for various benign and malignant tumors, particularly rhabdomyosarcoma. Clinical features are characteristic coarse facial features with relative macrocephaly, wide nasal bridges, loose skin, increased pigmentation with age, deep palmar and plantar creases with hyperkeratosis, papillomata of the face or perianal region, premature aging and hair loss, multifocal atrial tachycardia, pulmonic valve stenosis, hypertrophic cardiomyopathy, moderate psychomotor retardation and short stature. Skeletal abnormalities are common,

especially ulnar deviation at wrist, hyperextensibility of small finger joints, joint laxity, chest deformity, and cervical kyphosis. Additional features are polyhydramnios, large birth weight for gestational age, and severe feeding difficulty. The diagnosis is usually based on the recognition of distinctive clinical features. DNA testing is helpful for the confirmation of diagnosis. The mutation is most commonly (80-90%) identified in the *HRAS* gene. Growth failure is serious. Adult height ranges 122-154 cm in females, 124-153 cm in males. The pathophysiology underlying growth failure is not clearly understood. GH secretion is reported to be impaired in some cases, and feeding and nutritional issues may contribute to growth failure. It is unclear whether or not GH therapy is beneficial in Costello syndrome. Even if growth hormone deficiency is present, GH therapy should be cautiously prescribed. Hypertrophic caridiomyopathy might be worsened and malignancy (ex. bladder carcinoma) was reported with GH therapy. Special education and early intervention are needed because of high prevalence of mental retardation (IQ usually ranges 30-87). Cardiac abnormalities are present in 80% of patients. Hypertrophic cardiomyopathy is most common (60%). Congenital heart defect is present in 30% of CS patients, interestingly involving pulmonic or mitral valve or polyvalves. Respiratory care is also important because laryngomalacia, tracheomalacia, and bronchomalacia are common. Foot problem and kyphoscoliosis are common, frequently necessitating surgical intervention. EEG abnormalities are observed in 75% of patients and seizure occurs in 11%, requiring anticonvulsants. In addition to growth problem, other endocrine issues are abnormal glucose homeostasis leading to hypoglycemia, and delayed puberty. Ophthalmologic evaluation must be done. Tumor surveillance is critical in CS patients. Ultrasound evaluation of abdomen and pelvis is necessary every 3-6 months until age 8-10 years to identify rhabdomyosarcoma

and abdominal neuroblastoma. Urine analysis for hematuria is needed annually to detect bladder carcinoma. Skin should be regularly examined for tags, warts, dry skin and acanthosis nigricans. Recurrent papilloma can be removed by dry ice [6, 22, 31, 42].

Conclusions

Noonan syndrome and its related disorders are not rare as a whole. Since their disease natural course and management are different, it is important to recognize RASopathies and differentiate them primarily based on typical clinical feature. By utilizing DNA testing, the confirmatory diagnosis can be made. Multi-systemic involvement in RASopathies requires multidisciplinary evaluation and regular monitoring for each special clinical issue. However, further research on molecular defects of the disease and optimal care guidelines for these patients remain to be developed.

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Table 1. Diverse clinical features of Noonan syndrome (modified from ref. 31)

Systems involved	Clinical features
Inheritance	Autosomal dominant(14-75%)
Growth	Short stature (postnatal onset) (50-80%) Failure to thrive in infancy (40%)
Head and neck	Triangular face Ear abnormalities (44-90%) : low-set posteriorly rotated ears and thick helix Eye abnormalities(95%) : ptosis, hypertelorism, epicanthal folds, down-slanting palpebral fissures, strabismus, proptosis, myopia and nystagmus Deeply grooved philtrum with high peaks of upper lip vermilion border(95%) Neck abnormalities (95%) : short or webbed neck High-arched palate (45-34%) Dental malocclusion (35%) Low posterior hairline (32%) Micrognathia (22%)
Cardiovascular	Congenital heart defect (50-75%) : pulmonary valve stenosis(50%), hypertrophic cardiomyopathy (10%), atrial septal defect (10%) and other (aortic stenosis, ventricular septal defects and mitral insufficiency) Electrocardiogram with left axis deviation and a negative pattern in the left precordial leads
Chest	Thoraxic abnormalities(53-70%) : flat, funnel, shield or deformed chest, pectus carinatum superiorly and/or pectus excavatum inferiorly
Genito-urinary	Cryptorchidism (60-69%) Puberty delay
Skeletal	Cubitus valgus (47%) Hand abnormalities : clinodactyly and brachydactyly and blunt fingertips (30%) Vertebral abnormalities (25%)
Neurologic	Motor developmental delay (26%), language delay (20%) and learning disability (15%) Mental retardation, generally mild (25-35%)
Hematology	Bleeding anomalies(20%), including factor XI or XII deficiencies, von Willebrand's disease, platelet dysfunction and leukemia (in especial juvenile myelomonocytic leukemia-JMML) , transient myeloproliferative disorder
Other	Peripheral lymphedema, splenomegaly, deafness, increased cancer risk Malignant hyperthermia by anesthetics

Values in parentheses show percent frequency.

Table 2. Genotype and phenotype correlations among Korean patients with NS and NS-related disorders (cited from ref.22)

	N/Total (%) of subjects						
	PTPN11 (n = 25)	*SOS1* (n = 12)	*RAF1* (n = 4)	*HRAS* (n = 5)	*BRAF* (n = 8)	*KRAS* (n = 3)	*SHOC2* (n = 4)
M:F	16:9 (64.0%:36.0%)	7:5 (58.3%:41.7%)	3:1	2:3	3:5	3:0	2:2
Cardiac	18/24 (75.0%)	10/12 (83.3%)	4/4	5/5	7/8	3/3	3/3
Pulmonic stenosis	11/24 (45.8%)[a]	10/12 (83.3%)[a-d]	0/4[b]	1/5[c]	6/8	1/3	0/3[d]
Hypertrophic cardiomyopathy	5/24(20.8%)[e]	2/12 (16.7%) [f]	4/4[e,f]	3/5	3/8	0/3	1/3
Ventricular septal defect	3/24 (12.5%)	3/12 (25.0%)	1/4	0/5	1/8	0/2	1/3
Atrial septal defect	6/24 (25.0%)	4/12 (33.3%)	1/4	1/5	3/8	1/3	2/3
Patent ductus arteriosus	7/24 (29.2%)	0/12 (0.0%)	0/4	0/5	0/8	0/3	0/3
Chest wall deformity	6/24 (25.0%)	7/12 (58.3%)	1/4	1/5	3/8	2/3	0/4
Cryptorchidism	7/17 (41.2%)	3/7 (42.9%)	1/3	1/2	2/3	0/3	0/2
SNHL	5/24 (20.8%)	0/11 (0.0%)	0/4	1/5	1/8	0/3	0/4
JMML	2/24 (8.3%)	0/12 (0.0%)	0/4	0/5	0/8	0/3	0/4
Bleeding tendency	4/24 (16.7%)	0/12 (0.0%)	0/4	1/5	0/7	0/3	0/4
Growth and development							
Short stature (<3rd)	17/23 (73.9%)	5/12 (41.7%)[g]	4/4	5/5[g]	6/8	1/2	4/4
Height (SDS)	-2.6 ± 1.87 (-6.40–2,85)	-1.94 ± 1.37 [h] (-5.11–0.61)	-2.31 ± 0.97 (-3.11 to -1.23)	-3.87 ± 1.96 [h] (-5.97 to -0.85)	-1.60 ± 2.48 (-4.40–2.63)	nd	-3.45 ± 0.53 (-.387 to -2.71)
IGF1 (SDS)	-1.72 ± 0.88 (-3.13 to -0.83)	-2.68 ± 0.89 (-3.93 to -1.66)	nd	nd	nd	nd	nd
IGF-BP3 (SDS)	-3.38 ± 2.19 (-8.92 to -0.34)	-2.83 ±1.89 (-5.41 to -0.89)	nd	nd	nd	nd	nd
Bone age delay (yr)	2.47 ± 0.70 (1.00–3.60)	0.82 ± 1.77 (-1.90–3.00)	nd	nd	nd	nd	nd
Global developmental delay /mental retardation	6/21 (28.6%) [i,j,k,l]	0/12 (0.0 %) [m-p]	2/4	5/5 [i,m]	7/8 [i,n]	3/3[k,o]	3/3[l,p]

[a-p]$P<0.05$; n.d., not-determined.

Figure 1. Distribution of genetic locus where the mutation is present in Korean patients with RASopathies

Figure 1.