Noonan Spectrum and RASopathies Panel (19 genes)

Disorder also known as: Noonan Spectrum disorders; Ras/MAPK pathway related disorders

Panel Gene List: PTPN11; SOS1; RAF1; KRAS; HRAS; BRAF; MAP2K1 (MEK1); MAP2K2 (MEK2); SHOC2; NRAS; CBL; RIT1; SPRED1; ACTB; ACTG1; A2ML1; KAT6B; LZTR1; SOS2

Clinical Features:

Noonan syndrome: Individuals with Noonan syndrome (NS) have dysmorphic facial features, which may include hypertelorism, downward slanting eyes, epicanthal folds, and low-set and posteriorly rotated ears. A variety of cardiac defects may be present, including pulmonary stenosis, patent ductus arteriosus, hypertrophic cardiomyopathy, and coarctation of the aorta. Other features include short stature, pterygium colli, short, webbed neck, deafness, motor delay, and bleeding diathesis. Lymphedema may be present prenatally. Noonan syndrome shares some clinical features with cardio-facio-cutaneous (CFC) and Costello syndrome; however, Noonan syndrome patients typically have milder cognitive deficits and fewer ectodermal problems.

Noonan-like syndrome with loose anagen hair: Individuals with this specific phenotype exhibit features consistent with Noonan syndrome as well as growth hormone deficiency, cognitive deficits, distinctive hyperactive behavior, loose anagen hair, darkly pigmented skin with eczema or scaling, and often mitral valve and septal cardiac defects.

Noonan syndrome with multiple lentigines: Previously known by the acronym of LEOPARD, which is derived from a number of features seen in this condition: multiple *L*entigines, *E*CG abnormalities, *O*cular hypertelorism, *P*ulmonary stenosis, *A*bnormal genitalia, growth *R*etardation, and sensorineural *D*eafness. The diagnostic criteria for Noonan syndrome with multiple lentigines are met when an individual has multiple lentigines and two other related features, or three related features and a first degree relative with multiple lentigines.

Cardio-facio-cutaneous (CFC) syndrome: CFC is characterized by cardiac defects, ectodermal abnormalities, developmental delay and facial dysmorphism. The ectodermal findings include sparse, slow-growing, curly hair, dry skin or ichthyosis, hyperkeratosis of the palms and soles, keratosis pilaris, eczema, hemangiomas, and hyperelastic skin. Typical dysmorphic features are macrocephaly, prominent forehead, hypertelorism, bitemporal constriction, posteriorly rotated ears, short bulbous nose with anteverted nares, hypoplastic supraorbital ridges, ptosis, and downslanting palpebral fissures. Webbing of the neck is seen in half the patients and cryptorchidism is observed in 43% of affected males. Ninety percent of patients have mental retardation (MR) ranging from mild to severe, with the majority having

moderate MR. Short stature and postnatal growth deficiency are seen in 80% of patients, and failure to thrive is a common manifestation in early life. Congenital heart defects, the most frequent of which are pulmonic stenosis, atrial septal defects and hypertrophic cardiomyopathy, are seen in 80% of affected individuals.

Costello syndrome: This complex developmental disorder is characterized by short stature, mental retardation, facial dysmorphism, cardiovascular abnormalities, musculoskeletal abnormalities and tumor predisposition. Costello syndrome may present in utero with polyhydramnios, edema, and fetal overgrowth. After birth, feeding difficulties, failure to thrive, and mild to moderate developmental and growth delay are common. The facial features are typically coarse, with a wide forehead, epicanthal folds, depressed nasal bridge, low-set ears with large, thick lobes, and thick lips. About 63% of patients have cardiovascular malformations, most commonly pulmonic stenosis, hypertrophic cardiomyopathy, and tachyarrhythmia.[18] The hair may be curly and the skin dark-colored, soft and lax, especially on the neck, palms and soles. Hands and feet are fleshy with deep palmar and plantar creases, and hyperextensible digits. During childhood, patients progressively develop benign skin tumors (papillomata) around the mouth, nose and anus. While these tumors are benign, 10%-15% of patients with Costello syndrome will also develop malignant tumors, such as rhabdomyosarcoma, neuroblastoma, ganglioneuroblastoma, and transitional carcinoma of the bladder.

Legius syndrome: Neurofibromatosis 1-like syndrome, or Legius syndrome, is an autosomal dominant disorder resembling neurofibromatosis 1 with cafe-au-lait spots, axillary freckling, macrocephaly, learning disabilities, ADHD, developmental delays, and dysmorphic facial features similar to Noonan syndrome.[27,28] Other typical NF1 features such as Lisch nodules of the iris, neurofibromas and central nervous system tumors are systematically absent.[27] Two studies revealed that approximately 2% of individuals fulfilling diagnostic criteria for NF1 have SPRED1 pathogenic variants.[32, 34]

Other phenotypically overlapping syndromes: In patients exhibiting dysmorphic features in combination with cardiac abnormalities (e.g. atrial septal defect, ventricular septal defect) and who test negative for RASopathies, other syndromes with variable phenotypic spectrums including milder presentations may be considered. Baraitser-Winter syndrome is a rare autosomal dominant congenital disorder shares a few overlapping features with Noonan spectrum disorders, although patients with full-blown disease have distinct characteristics. Patients may have fetal cystic hygroma/nuchal redundancy and develop a webbed neck, short stature, ptosis, hypertelorism and developmental delay. Classic Baraitser-Winter syndrome is characterized by ptosis, high-arched eyebrows, hypertelorism, ocular colobomata and anterior predominant lissencephaly.[44] Other common features are postnatal short stature, microcephaly, intellectual disability, seizures and hearing loss.[44] A Noonan-like phenotype

consisting of short stature, blepharoptosis, and intellectual disabilities has also been linked to haploinsufficiency of KAT6B.[49] Genitopatellar syndrome is a rare autosomal dominant congenital disorder characterized by hypoplastic or absent patellae, intellectual disability, craniofacial defects, genital anomalies, and congenital heart defects among others.[50,51] Say-Barber-Biesecker-Young-Simpson syndrome (SBBYSS) is characterized by narrowing of the palpebral fissures, intellectual disability, structural heart defects, hypoplastic patellae, joint laxity, severe hypotonia, feeding difficulty, immobile mask-like face and other anomalies.[48]

Genetics:

Noonan syndrome and the RASopathies belong to a genetically heterogeneous group of autosomal dominant disorders related to gain-of function effects in the Ras/MAPK signaling pathway. Baraitser-Winter syndrome, Genitopatellar syndrome and Say-Barber-Biesecker-Young-Simpson syndrome (SBBYSS) are also autosomal dominant disorders with some phenotypic overlap including dysmorphic features and cardiac abnormalities. Most cases of these disorders are sporadic due to a *de novo* variant; however, familial cases, particularly of Noonan syndrome and rarely of CFC syndrome have been described.

Test Methods:

The coding regions and splice junctions of the 19 genes of this panel are enriched using a proprietary targeted capture system developed by GeneDx. The targeted regions are sequenced simultaneously by massively parallel (NextGen) sequencing on an Illumina platform with paired-end reads. Bi-directional sequence is assembled, aligned to reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed for sequence variants. Capillary sequence is used to confirm all potentially pathogenic variants and to obtain sequence for regions where fewer than 19 reads are achieved by NextGen sequencing.

Test Sensitivity:

Noonan syndrome: Approximately 88.5% of individuals with a clinical diagnosis of Noonan Syndrome are expected to have a heterozygous pathogenic variant in one of six genes, PTPN11, SOS1, RAF1, RIT1, NRAS, CBL, or KRAS, which encode proteins with distinct roles in intracellular RAS-MAPK signaling. PTPN11 pathogenic variants have been found in approximately 50% of affected individuals, with a higher rate (59%) in familial vs. sporadic (37%) cases. Germline pathogenic variants in SOS1 are responsible for NS in about 10% of Noonan Syndrome patients, and 20% of those who do not have a pathogenic variant in PTPN11. Germline RAF1 pathogenic variants occur in as many as 17% of NS patients, and ~7% - 33% of those without a pathogenic variant in PTPN11, SOS1 or KRAS. Germline pathogenic variants in KRAS account for approximately 1-2% of all NS cases. Germline pathogenic variants in NRAS and CBL account for approximately 0.5% and 1%, respectively, of all NS cases. The overall frequency of RIT1 pathogenic variants in individuals with a diagnosis of Noonan syndrome has not been well established. Based on current studies in

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Japanese and Brazilian cohorts, RIT1 pathogenic variants were found in approx. 9% of NS patients who tested negative for a pathogenic variant in other known NS genes.[37,40] SOS2 pathogenic variants have been found in approximately 4% of affected individuals who were previously negative for variants in the most common Noonan Syndrome genes.[47] LZTR1 pathogenic variants have been found in approximately 8% of affected individuals who were previously negative for variants in the most common Noonan Syndrome genes.[47] A2ML1 pathogenic variants have been found in approximately 1% of affected individuals who were previously negative for variants in the known Noonan Syndrome genes.[46] Genotype-phenotype correlations suggest that PTPN11 pathogenic variants are often associated with pulmonic stenosis (70%). In contrast, about 80% of NS patients with a pathogenic variant in the RAF1 gene can be expected to develop hypertrophic cardiomyopathy. Certain RAF1 pathogenic variants almost invariably appear to result in HCM. Patients with pathogenic variants in CBL or PTPN11 may have an increased risk for developing juvenile myelomonocytic leukemia (JMML).

Noonan-like with loose anagen hair: The Ser2Gly pathogenic variant in the SHOC2 accounts for approximately 5% of all patients with a Noonan-like phenotype and negative results for analysis of the PTPN11, SOS1, RAF1, and KRAS genes. This pathogenic variant typically has a distinct clinical presentation, including features of Noonan syndrome and loose anagen hair, in association with one or more of the following features: distinctive hyperactive behavior, mitral valve dypslasia and septal cardiac defects.

Noonan syndrome with multiple lentigines: Germline pathogenic variants in PTPN11 have been found in almost 90% of patients with Noonan syndrome with multiple lentigines. In one recent study, 2 out of 6 patients with a clinical diagnosis of Noonan syndrome with multiple lentigines and hypertrophic cardiomyopathy harbored a pathogenic variant in the RAF1Gene.

Cardio-Facio-Cutaneous syndrome: In CFC, a study of 56 patients revealed an overall pathogenic variant detection rate of over 62% when BRAF, MAP2K1, MAP2K2 and KRAS were analyzed.

Costello syndrome: 82% to 92% of patients with Costello syndrome are expected to have a missense pathogenic variant in the HRAS gene.

Legius syndrome: SPRED1 pathogenic variants have been reported in approximately 2% of individuals who meet NIH NF1 diagnostic criteria, and in 3-25% who met NF1 criteria and were negative for an NF1 gene pathogenic variant.[28,31,32] SPRED1 pathogenic variants have also been reported in approximately 1.3% of individuals with a clinical diagnosis of NF1 and who have had no previous genetic testing.[34] However, the sensitivity of SPRED1 sequencing for the cohort discussed by Muram-Zborovski et al. increased to 20% if patients with an affected

parent, optic pathway tumor, Lisch nodules, neurofibromata, long bone dysplasia, or sphenoid wing dysplasia were excluded.[34] Deletions of the SPRED1 gene account for approximately 10% of all pathogenic variants identified in the SPRED1 gene.[35] The frequency of SPRED1 pathogenic variants among patients with suspected Noonan syndrome is currently unknown.

Other phenotypically similar syndromes: In a cohort of 42 patients diagnosed with Baraitser-Winter syndrome 79% of patients harbored a variant within the ACTB gene while the remaining 21% harbored a variant within the ACTG1 gene.[45] In a cohort of 19 individuals diagnosed with Say-Barber-Biesecker-Young-Simpson syndrome (SBBYSS) 74% of patients harbored a variant in the KAT6B gene.[48] Of individuals diagnosed with Genitopatellar syndrome (GPS) 83-100% harbor a variant in the KAT6B gene.[50,51s]

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