

Noonan and Comprehensive RASopathies Panel (25 genes)

DISORDER ALSO KNOWN AS

Noonan Spectrum disorders; Ras/MAPK pathway related disorders, RASopathy

PANEL GENE LIST

A2ML1, ACTB, ACTG1, BRAF, CBL, HRAS, KAT6B, KRAS, LZTR1, MAP2K1, MAP2K2, NF1, NRAS, NSUN2, PPP1CB, PTPN11, RAF1, RASA1, RASA2, RIT1, RRAS, SHOC2, SOS1, SOS2, SPRED1

CLINICAL FEATURES OF RASOPATHIES

Noonan syndrome: Noonan syndrome (NS) is a disorder involving defects in the Ras/MAPK pathway. It is characterized by dysmorphic features, short stature, developmental delays, bleeding issues and cardiac defects, however, there is a wide phenotypic spectrum. Dysmorphic facial features may include hypertelorism, downward slanting eyes, epicanthal folds, and low-set and posteriorly rotated ears.¹ One or more cardiac defects may be present including pulmonary stenosis, patent ductus arteriosus, hypertrophic cardiomyopathy, and coarctation of the aorta. Other features may include pterygium colli, webbed neck, deafness, and prenatal lymphedema.² 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.^{1,3}

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.^{2,4}

Noonan syndrome with multiple lentiginos: This condition was previously known as LEOPARD (multiple **L**entiginos, **E**CG abnormalities, **O**cular hypertelorism, **P**ulmonary stenosis, **A**bnormal genitalia, growth **R**etardation, and sensorineural **D**eafness) syndrome. The diagnostic criteria for Noonan syndrome with multiple lentiginos are met when an individual has multiple lentiginos and two other related features, or three related features and a first degree relative with multiple lentiginos.²

Cardio-facio-cutaneous (CFC) syndrome: CFC is characterized by cardiac defects, ectodermal abnormalities, developmental delay and facial dysmorphism. 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 include 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 of all 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 are seen in 80% of affected individuals with pulmonic stenosis, atrial septal defects and hypertrophic cardiomyopathy most frequently observed.^{1,2}

Costello syndrome: This 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. Facial features are typically coarse, with a wide forehead, epicanthal folds, depressed nasal bridge, low-set ears with large, thick lobes, and thick lips.^{1,5,6} About 63% of patients have cardiovascular malformations, most commonly pulmonic stenosis, hypertrophic cardiomyopathy, and tachyarrhythmia.⁶ Hair may be curly and skin may be 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. In addition to these benign tumors, approximately 10%-20% of patients with Costello syndrome will develop malignant tumors, such as rhabdomyosarcoma, neuroblastoma, ganglioneuroblastoma, and transitional carcinoma of the bladder.^{1,5}

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.⁷ Other typical NF1 features including Lisch nodules of the iris, neurofibromas and central nervous system tumors are absent.^{1,7} Two studies revealed that approximately 2% of individuals fulfilling diagnostic criteria for NF1 have pathogenic variants in *SPRED1*.^{8,9}

Other Ras/MAPK pathway or phenotypically overlapping syndromes: Pathogenic variants in another Ras/MAPK pathway gene *RASA1* cause Parkes Weber syndrome or Capillary Malformation-Arteriovenous Malformation syndrome (CV-AVM), which are rare autosomal dominant disorders characterized by arteriovenous malformations.¹ In patients who exhibit dysmorphic features in combination with cardiac abnormalities (e.g. atrial septal defect, ventricular septal defect) and 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 that 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.¹⁰ Other common features are postnatal short stature, microcephaly, intellectual disability, seizures and hearing loss.¹⁰ A Noonan-like phenotype consisting of short stature, blepharoptosis, and intellectual disabilities has also been linked to haploinsufficiency of *KAT6B*.¹¹ 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.^{12,13} 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.¹⁴

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 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.

Loss-of-function *NSUN2* and *RASA2* variants have been associated with Noonan-like syndrome in several individuals.^{20,27,28} Missense *PPP1CB* variants have been identified in several individuals with clinical features of Noonan syndrome and loose anagen hair.^{30,31} Rare gain-of-function or biallelic loss-of-function *LZTR1* variants have been associated with Noonan syndrome, while historically heterozygous loss-of-function variants have been associated with a predisposition to schwannomatosis.³⁸

TEST METHODS

Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the genes on this panel are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons. For the *HRAS* gene, sequencing but not deletion/duplication analysis is performed. Only large whole exon deletion/duplication events are detectable for *ACTB*.

Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

Test Sensitivity

While each RASopathy has a unique phenotype, there are many overlapping characteristics and molecular heterogeneity. Additional information about the general clinical sensitivity of each gene for different clinical disorders is included in the table below.

Gene	Noonan or Noonan-like syndrome	Noonan syndrome with multiple lentigines	CFC syndrome	Costello syndrome	NF1 syndrome	LEgus syndrome	CV-AVM
<i>A2ML1</i>	~1% ^{20,25}	--	--	--	--	--	--
<i>BRAF</i>	< 2% ³⁷	--	75% ¹	--	--	--	--
<i>CBL</i>	~1% ^{18,19,20}	--	--	--	--	--	--
<i>HRAS</i>	--	--	--	82-92% ^{1,5,32}	--	--	--
<i>KRAS</i>	~1-2% ²⁰	--	< 2-5% ²	--	--	--	--
<i>LZTR1</i>	~8% ^{20,25}	--	--	--	--	--	--
<i>MAP2K1 (MEK1)</i> <i>MAP2K2 (MEK2)</i>	--	--	~25% ¹	--	--	--	--
<i>NF1</i>	--	--	--	--	> 90% ²	Unknown	--
<i>NRAS</i>	< 1% ^{18,19,20}	--	--	--	--	--	--
<i>NSUN2</i>	Unknown ^{27,28}	--	--	--	--	--	--
<i>PPP1CB</i>	Unknown ^{30,31}	--	--	--	--	--	--
<i>PTPN11</i>	50% ²⁰	90% ²⁷	--	--	--	--	--
<i>RAF1</i>	~5-10% ²⁰	Unknown ²⁷	--	--	--	--	--
<i>RASA1</i>	--	--	--	--	--	--	95% ³⁶
<i>RASA2</i>	Unknown ²⁰	--	--	--	--	--	--
<i>RIT1</i>	5-10% ^{21,22,23}	--	--	--	--	--	--

<i>RRAS</i>	< 1% ^{20,26}	--	--	--	--	--	--
<i>SHOC2</i>	< 1% ²⁰	--	--	--	--	--	--
<i>SOS1</i>	~10-15% ^{15,16,20}	--	--	--	--	--	--
<i>SOS2</i>	~4% ^{20,25}	--	--	--	--	--	--
<i>SPRED1</i>	Unknown ^{7,8,33}	--	--	--	Unknown ^{7,8,33}	3-25% ^{7,8,33}	--

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.³⁵ 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.¹⁴ Of individuals diagnosed with Genitopatellar syndrome (GPS) 83-100% harbor a variant in the *KAT6B* gene.^{12,13}

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