Medical Genomics Laboratory
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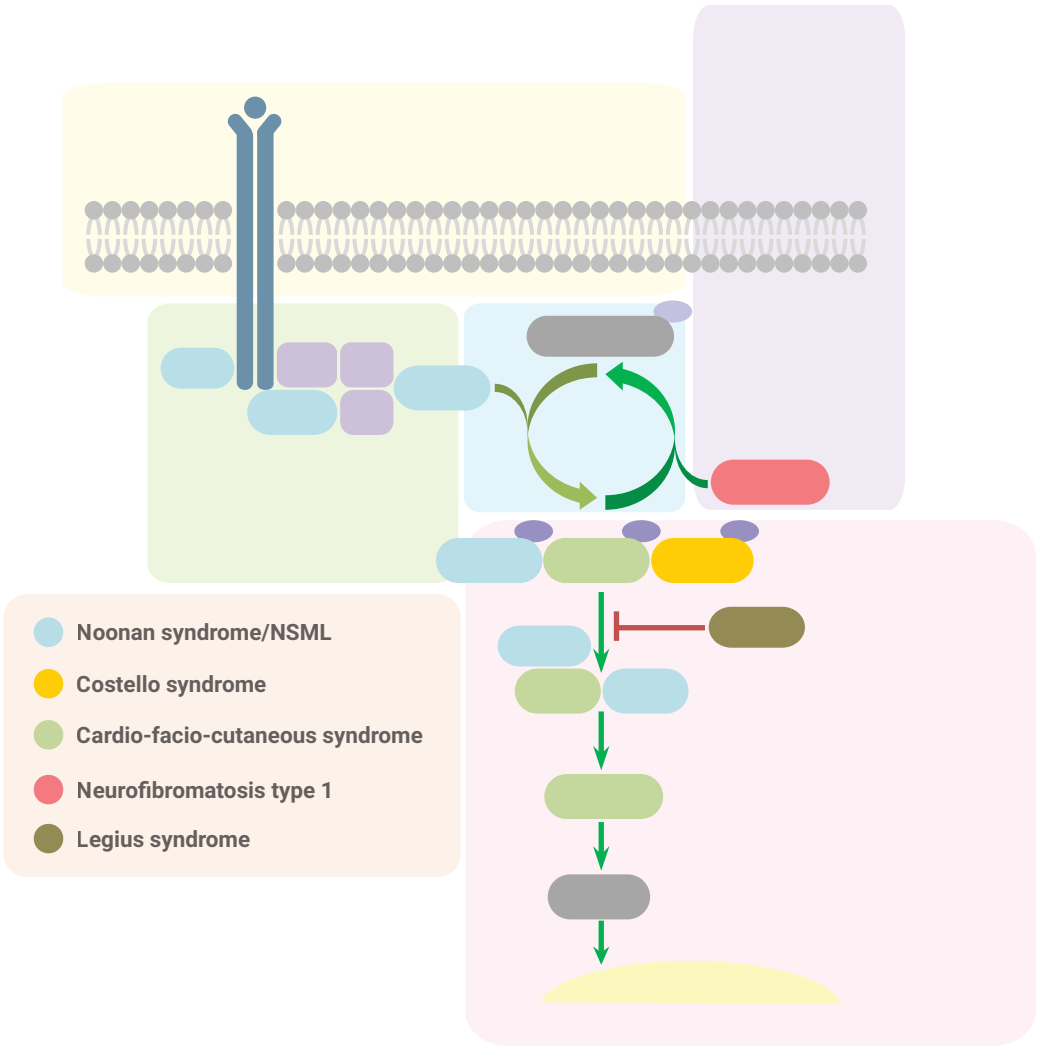

Comprehensive testing for neurofibromatoses, RASopathies, and tuberous sclerosis at UAB

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TABLE OF CONTENTS

DISORDER BACKGROUND

Neurofibromatosis type 1 2
Legius syndrome 2
Noonan syndrome 2
Cardio-Facio-Cutaneous syndrome 3
Costello syndrome 3
Capillary/Arteriovenous Malformation syndrome/Parkes Weber 3
McCune-Albright syndrome *(GNAS)* 3
Neurofibromatosis type 2 4
Schwannomatosis/Multiple Schwannomas 4
Meningiomatosis/Multiple Meningiomas 4
Peripheral Nerve Sheath Tumors 5
Rhabdoid Tumor Predisposition syndrome 5
Tuberous Sclerosis Complex 5

TESTING OPTIONS

DNA-based Testing via NGS

NF1-only by NGS (NF1-NG) 7
NF1/SPRED1 by NGS (NFSP-NG) 8
Expanded *NF1*-RASopathy NGS Panel (RAS-NG) 8
Non-*NF1* RASopathy NGS Panel (NNP-NG) 9
Costello syndrome *HRAS*-only by NGS (CST-NG) 9
Capillary/Arteriovenous Malformation syndrome/Parkes Weber by NGS (RASA-NG) 9
McCune-Albright syndrome *GNAS*-only by NGS (GNAS-NG) 10
NF2-only by NGS (NF2-NG) 10
Schwannomatosis/Multiple Schwannomas Panel by NGS (SCH-NG) 10
Meningiomatosis/Multiple Meningiomas Panel by NGS (MEN-NG) 10
Peripheral Nerve Sheath Tumor Panel by NGS 11
Rhabdoid Tumor Predisposition syndrome by NGS (RT-NG) 11
Tuberous Sclerosis Complex Panel by NGS (TSCP-NG) 12

RNA-based Testing via Sanger

RNA-based *NF1*/DNA-based *SPRED1* testing on blood (NFSP-R) 12
RNA-based *NF1* testing on blood (NF1-R) 13
RNA-based *NF1*/DNA-based *SPRED1* testing on cultured cells from affected tissues (NF14C/NF14N) 13

Additional Information

Variant Confirmation Policy 14
References 14

TESTING OPTIONS OVERVIEW

A comprehensive menu allowing a tailored approach for patients with constitutional or mosaic presentations

TEST TARGET	ACCEPTABLE SPECIMEN
DNA-based Testing by NGS	
• *NF1*-only* • *NF1/SPRED1** • Expanded *NF1*-RASopathy Panel (18 genes)* • Non-*NF1* RASopathy Panel (17 genes)* • Costello Syndrome (*HRAS*) • Capillary/Arteriovenous Malformation syndrome/Parkes Weber(2 genes)* • *GNAS*-only	Blood Saliva Extracted DNA Targeted Sperm Analysis
• Peripheral Nerve Sheath Tumor Panel (6 genes)* • *NF2*-only* • Schwannomatosis/Multiple Schwannomas Panel (3 genes)* • Meningiomatosis/Multiple Meningiomas Panel (4 genes)* • Tuberous Sclerosis Complex (2 genes)* • Rhabdoid Tumor Predisposition Syndrome (2 genes)*	Blood Saliva Extracted DNA Fresh/Frozen Tumor Targeted Sperm Analysis
RNA-based Testing by Sanger	
• *NF1*-only* • *NF1* with *SPRED1* gDNA analysis*	Fresh Blood
• *NF1*-only* • *NF1* with *SPRED1* gDNA analysis*	Fresh Biopsy
DNA-based Tumor Testing by Sanger	
• *NF2*-only* • Schwannomatosis/Multiple Schwannomas Panel (3 genes)* • Rhabdoid Tumor Predisposition Syndrome (*SMARCB1*)*	Formalin-fixed Paraffin-Embedded Tumor (FFPE)

* Copy number analysis is included for the gene(s) in this panel. See test description for more information.

❖ *The RASopathies*

The RASopathies are a genetically heterogeneous group of disorders caused by variants in the genes involved in the Ras-MAPK pathway. As a group, the RASopathies are one of the largest groups of malformation syndromes known, affecting ~1:1,000 individuals and include neurofibromatosis type 1, Legius syndrome, Noonan syndrome, cardio-facio-cutaneous (CFC) syndrome, Noonan syndrome with multiple lentigines (NSML/LEOPARD) and Costello syndrome. Variants in ***NF1*** and ***SPRED1*** are typically loss-of-function variants and include the full spectrum of nonsense, missense, splice, frameshift, insertion-deletion, and copy number changes. Variants in the other RASopathy genes are typically missense variants and/or in-frame deletion/insertion of an amino acid.

The genes within the Ras/MAPK pathway can have a profound deleterious effect on development due to their key role in differentiation, growth, senescence, and dysregulation. Clinical features of the RASopathies include short stature; cardiovascular defects; cutaneous and pigmentary findings; characteristic facies; skeletal and neurocognitive delays as well as a predisposition to neoplasia, both benign and malignant. The RASopathies are inherited in an autosomal dominant manner. A parent who carries a mutated gene has a 50% chance of passing it on to every child, regardless of gender. The disorders have variable expressivity (individuals with the same disorder may show different features and severity of symptoms, even within the same family). Some of the genes/variants are not fully penetrant, therefore an individual may carry a variant and only show few to no signs of the syndrome. Moreover, features can change/progress with age, which makes it difficult to make an accurate clinical diagnosis. An individual can carry a variant either a) because (s)he inherited the variant from a parent (parent clinically affected or "non-penetrant"), or b) because the variant arose "de novo" in the egg or sperm from which the individual developed. Sometimes, the variant occurred "post-zygotically", i.e. during development and in these individuals the variant may not be present in every cell of the body, typically resulting in a milder phenotype (mosaicism).

Neurofibromatosis type 1

The ***NF1*** gene, cloned in 1990, was the first gene within the Ras-MAPK pathway shown to be associated with an autosomal dominant disorder, **Neurofibromatosis type 1 (NF1)**. NF1 affects ~1/3000 individuals worldwide, with half of the patients resulting from a de novo event within their family (sporadic). NF1 is notorious for its phenotypic variability and is a progressive disorder with more clinical signs developing with time. Although the NIH criteria enable clinicians to make a diagnosis in the majority of classically affected cases, diagnostic criteria are often not met until a given age is reached. Atypical presentations also exist with patients not yet fulfilling NIH criteria by adulthood. The mutational spectrum of NF1 is very complex and includes a wealth of unusual splice variants affecting exonic sequences as well as deep intronic variants resulting in exonization of intronic sequences at the mRNA level.

Legius syndrome

Germline loss-of-function variants in ***SPRED1***, a negative regulator of the RAS-MAPK pathway, cause a neurofibromatosis type 1-like phenotype, first described in 2007 (**Legius syndrome**). Patients present with multiple café-au-lait spots with or without skinfold freckling. Other typical NF1 associated features (Lisch nodules, bone abnormalities, neurofibromas, optic pathway gliomas) are systematically absent. However, in some patients Noonan-like features are present.

BACKGROUND

Noonan syndrome

Noonan syndrome (NS), **Noonan syndrome with multiple lentigines** (NSML, aka LEOPARD) and **Noonan syndrome with "loose anagen hair"** are autosomal dominant disorders affecting ~1:1,000-2,000 individuals. Patients present with craniofacial features and a variable clinical phenotype including congenital heart defects, reduced growth, bleeding disorders (NS), and varying degrees of neurocognitive delay. Patients with NSML also have multiple lentigines, genital abnormalities, and sensorineural deafness. Patients with NS also have an increased cancer predisposition. Genes associated with NS and NSML are ***PTPN11, PPP1CB, KRAS, SOS1, RAF1, NRAS, BRAF, MAP2K1, CBL, RIT1, RASA2*** and ***SOS2***. The ***SHOC2*** and ***PPP1CB*** genes are associated with NS with "loose anagen hair" or sparse slow growing hair (Gripp K. et al, Am J Med Genet. 2016;170(9):2237-2247).

Cardio-Facio-Cutaneous syndrome

Cardio-facio-cutaneous syndrome (CFC) is a rare condition with genetic and phenotypic overlap with NS. Clinical features include craniofacial features similar to those found in NS, neurocognitive delay, failure to thrive, congenital heart defects, epilepsy, and a wide range of ectodermal manifestations. Four genes have been associated with CFC: ***BRAF, MAP2K1, MAP2K2*** and ***KRAS***.

Costello syndrome

Costello syndrome (CS), caused by activating ***HRAS*** variants, is a very rare condition with the following key features: coarse facial features, severe feeding difficulty, mild to moderate intellectual disability, relative macrocephaly and short stature, and a high incidence of cardiac abnormalities and malignancy. Differentiation of CS from other RASopathies, particularly CFC, may be difficult especially early in life.

❖*Capillary/Arteriovenous Malformation syndrome/Parkes Weber syndrome*

Capillary/Arteriovenous malformation syndrome (CM-AVM) is a disorder of the vascular system. Parkes Weber syndrome is characterized by congenital vascular abnormalities known as capillary malformations and arteriovenous fistulas (AVFs). Some vascular abnormalities seen in Parkes Weber syndrome are similar to those that occur in capillary malformation-arteriovenous malformation syndrome (CM-AVM). CM-AVM and some cases of Parkes Weber syndrome are caused by variants in the ***RASA1*** and ***EPHB4*** genes. (Erola I et al, Am J Hum Genet. 2003;73:1240–9; Bayrak-Toydemir P, Stevenson D, Genereviews.2019).

❖*McCune-Albright Syndrome (GNAS)*

McCune-Albright Syndrome is caused by variants affecting codons p.Arg201 and p.Gln227 in exons 8 and 9 of *GNAS* and is characterized by polyostotic fibrous dysplasia, café-au-lait hyperpigmentation and precocious puberty (Boyce AM, et al. Genereviews. 2019). Patients with McCune-Albright syndrome have mosaic *GNAS* variants; thus the disorder presentation is highly variable, depending on the specific tissues involved and the extent of the involvement. No patients with non-mosaic gain of function GNAS variants are seen as such mutations are presumed to be lethal to an embryo. (Aldred and Trembath, 2000; Lumbroso et al., 2004). The MGL provdies testing for GNAS given the phenotypic overlap between segmental/mosaic NF1/Leigus syndrome and McCune-Albright syndrome.

❖*Schwannomatosis/ Multiple Schwannomas*

Schwannomas are nerve sheath tumors which are nearly always benign but can cause significant pain. Isolated schwannomas are common in the general population, but the development of multiple non-intradermal schwannomas (in the absence of bilateral vestibular schwannomas, congenital cataracts or ependymomas, typically associated with constitutional NF2) as typically seen in schwannomatosis patients, is rare. The presence of multiple non-intradermal schwannomas, in the absence of a family history of NF2, can be found in individuals with mosaic neurofibromatosis type 2, or in individuals carrying germline variants in either ***SMARCB1*** or ***LZTR1***.

Individuals with **schwannomatosis** do not typically develop bilateral vestibular schwannomas, ependymomas, meningiomas (typically associated with NF2), nor neurofibromas or astrocytomas (associated with NF1). However, significant clinical overlap with classic/mosaic neurofibromatosis type 2 exists: some *SMARCB1*-positive patients have developed meningiomas or a unilateral vestibular schwannoma; and some *LZTR1*-positive patients have been reported with a unilateral/bilateral vestibular schwannoma. Both NF2 and schwannomatosis present with variable expressivity; however, penetrance for NF2 is close to 100%, whereas non-penetrance is well documented in schwannomatosis, although the exact frequency is not known.

❖*Meningiomatosis/Multiple Meningiomas*

A **meningioma** in childhood can be a presenting first sign of neurofibromatosis type 2 (OMIM 101000) (Smith M et al, 2011: J. Med. Genet 48:261-5). Besides ***NF2***, germline ***SMARCB1*** variants have been identified in patients with meningiomas (with or without schwannomas) (Van den Munckhof P et al, 2012: Neurogenetics 13:1-7). Furthermore, germline variants in ***SUFU*** have been found in some families with **meningiomatosis** (Aavikko M et al, 2012: Am. J. Hum. Genet. 91:520-6).

SMARCE1 germline variants are associated with a tumor-predisposition syndrome with patients having an increased risk for spinal and intracranial clear cell meningiomas (Smith M et al, 2014: J. Path. 234:436-40). Clear cell meningiomas occur more frequently in young people and are defined as WHO grade 2, due to their more aggressive behavior.

As multiple meningiomas can be a sign of various tumor-predisposition syndromes that have overlapping features, a clinical diagnosis might be challenging in some individuals. A panel-based test is more cost-effective in these cases.

❖*Peripheral Nerve Sheath Tumors*

Peripheral nerve sheath tumors develop from Schwann cells, which are a type of cell that covers the peripheral nerves. These tumors can be benign or malignant, although ~90% of such tumors are benign. Nerve sheath tumors include **neurofibromas** and **schwannomas**. They can occur as solitary lesions; however, if multiple develop, this may indicate the presence of a hereditary predisposition.

Neurofibromas are common, benign tumors, composed of a complex heterogeneous mixture of cells, and the presence of 2 or more such tumors (cutaneous, subcutaneous or plexiform) is a diagnostic sign of neurofibromatosis type 1, with two ***NF1*** hits found specifically in the Schwann cells only. Whereas cutaneous neurofibromas never undergo malignant transformation, subcutaneous and plexiform neurofibromas may undergo malignant transformation (Evans DG, J Med Genet. 2002 May;39(5):311-4.).

BACKGROUND

Schwannomas are more homogeneously composed benign tumors and if multiple such tumors are present, they may indicate the presence of neurofibromatosis type 2 (NF2) or schwannomatosis. The location of the schwannomas differs between ***NF2*** and ***SMARCB1***- or ***LZTR1***-related schwannomatosis with (bilateral) vestibular schwannomas typically present in classic NF2, as well as intradermal and non-intradermal schwannomas. Schwannomatosis-associated schwannomas are usually non-intradermal and non-vestibular, however overlap clearly exists and genetic characterization can be an important tool to distinguish between both disorders.

Some types of schwannomas mimic neurofibromas and vice versa. They are called myxoid schwannomas and hybrid neurofibromas/schwannomas.

In addition, one patient with a ***KRAS*** germline variant p.K5E who presented with multiple diffuse schwannomas was described (Bertola DR, et al, Clin Genet. 2012;81(6):595-7). A study (Serrano C, et al, Histopathology. 2013;62(3):499-504) also found the *KRAS* variant p.G12S in 1/40 sporadic schwannomas.

Furthermore, several patients with Noonan syndrome with multiple lentigines (NSML), who fulfill criteria for NF1, and carry a variant in the ***PTPN11*** gene but not in the *NF1* gene, have recently been described. The patients had plexiform neurofibromas, large dumbbell spinal tumors and hypertrophic peripheral nerves.

❖*Neurofibromatosis Type 2*

Neurofibromatosis type 2 (NF2) is an autosomal dominant disorder characterized by bilateral vestibular schwannomas with associated symptoms of tinnitus, hearing loss, and balance dysfunction. Almost all affected individuals develop bilateral vestibular schwannomas by 30 years of age. Affected individuals may also develop schwannomas of other cranial and peripheral nerves, meningiomas, and ependymomas. ***NF2*** is the only gene in which pathogenic variants are known to cause neurofibromatosis type 2, however there is significant overlap between NF2 and schwannomatosis.

❖*Rhabdoid Tumor Predisposition syndrome*

Rhabdoid tumors are rare, aggressive childhood cancers that most often develop in the kidney (Malignant Rhabdoid Tumor, MRT) and central nervous system (Atypical Teratoid/Rhabdoid Tumor, AT/RT). These lesions can occur spontaneously or as part of hereditary **Rhabdoid tumor predisposition syndrome** (RTPS), associated with variants in ***SMARCB1*** or ***SMARCA4***. Compared to sporadic isolated rhabdoid tumors, the syndromic form is associated with an increased risk of developing multiple tumors at younger ages and schwannomas presenting primarily in adulthood. (Sevenet N. et al, 1999: Hum. Mol. Genet. 8:2359-68).

❖*Tuberous Sclerosis Complex*

Tuberous sclerosis complex (TSC) is a rare autosomal dominant disorder involving abnormalities of the skin, brain, kidney, heart and lungs. CNS tumors are seen commonly in patients with TSC. Heterozygous pathogenic variants can be identified in 75%-90% of individuals who meet the clinical diagnostic criteria for TSC (Northrup H. et al, 2013: Ped. Neurology 49:243-4). Among those in whom a pathogenic variant can be identified, pathogenic variants in ***TSC1*** and ***TSC2*** are found in 31% and 69% of cases, respectively (Sancak O et al. Eur J Hum Genet. 2005;13:731–41).

❖*Ambiguous Cases*

Some individuals with a clinical diagnosis of one of the neurofibromatoses and/or RASopathies have been found to carry a variant in a gene that was not considered to be consistent with their clinical diagnosis. Examples include *BRAF* variants reported in individuals with a clinical diagnosis of Noonan syndrome, a *SOS1* variant in an individual with CFC (Nystrom AM et al, J Med Genet. 2008;45(8):500-6), *PTPN11* variants in individuals with paraspinal neurofibromas (Conboy E. et al, J Med Genet. 2016;53(2):123-6), and an *NF1* missense variant in patients with Noonan-like features and no neurofibromas (Rojnueangnit K et al, Hum Mutat.2015;36(11);1052-63). In addition, some genes are associated with more than one syndrome (*PTPN11, KRAS, BRAF, RAF1, NF1*). Therefore, the comprehensive approach of simultaneously testing all 18 genes available in the Expanded NF1-RASopathy NGS panel (please see pg 8 for more detail) is best in some cases as it eliminates the need to determine which genes to test based on an individual's overlapping clinical signs.

Given the spectrum of genes involved in the RASopathies, NF2, schwannomatosis, tuberous sclerosis complex and the resulting phenotypic variability and /or overlap, the initial diagnosis for these conditions is often clinical. Molecular genetic testing is used for confirmatory diagnosis and assisting with diagnosis in ambiguous cases. Molecular diagnoses can play a significant role in helping to overcome the limitations of the clinical diagnoses with progressive accumulation of genotype–phenotype correlation data. Moreover, it can also aid in discovery of potential therapeutic targets and improve patient management.

❖*DNA-based Testing via NGS*❖

TEST OPTIONS

- *NF1*-only by NGS
- *NF1/SPRED1* by NGS
- Expanded *NF1*-RASopathy NGS Panel (18 genes)*
- Non-*NF1* RASopathy NGS Panel (17 genes)*
- Capillary/Arteriovenous Malformation syndrome/Parkes Weber (2 genes)
- Costello syndrome (*HRAS*)
- *GNAS*-only by NGS
- *NF2*-only by NGS*
- Schwannomatosis/Multiple Schwannomas Panel by NGS (3 genes)*
- Meningiomatosis/Multiple Meningiomas Panel by NGS (4 genes)*
- Peripheral Nerve Sheath Tumor Panel (6 genes)*
- Rhabdoid Tumor Predisposition syndrome (2 genes)*
- Tuberous Sclerosis Complex (2 genes)*

SPECIMEN TYPES

- Blood (3-6ml EDTA; no time limitations associated with receipt)
- Saliva (OGR-575 DNA Genotek; kits are provided upon request),
- DNA (extracted from lymphocyte cells, a minimum volume of 25µl at 3µg, O.D. value at 260:280nm ≥1.8)
- Fresh/Frozen (>60% pure tumor cells, 3-5mm-cubed in size) tumor/tissue testing available for any test notated with an astrisk (*)

TURNAROUND TIME

- 25 working days / 30 working days for tumor/tissue options
- RUSH option: 15 working days for an additional $600 USD

TESTING OPTIONS

NF1-only by NGS

Candidates for this test: patients with classic NF1 *including* the presence of cutaneous neurofibromas or Lisch nodules, as no genetic heterogeneity has been demonstrated so far associated with this phenotype.

The ***NF1*-only by NGS test** involves sequencing as well as **deletion/duplication analysis** of the entire coding ***NF1*** region plus the alternatively spliced exons 9br, 23a and 48a (60 exons total). The test uses an extensively customized and optimized set of Agilent HaloPlex capture probes, followed by sequencing of overlapping amplicons within the regions of interest using 300bp paired-end Illumina sequencing chemistry. Each coding exon plus ~50 bp of flanking intronic sequence is simultaneously sequenced. 5' and 3' untranslated sequences are not included. The **average coverage is >1600x with >98% of the *NF1* coding region covered at ≥350x and 99% at ≥200x, allowing detection of very low level mosaicism down to 3-5% variant allele fraction (regions covered by ≥350x and ≥200x respectively) with 95% confidence.** Variant and copy number calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels, and frameshifts caused by a deletion/duplication up to 112bp.

Based on >15 years of experience with comprehensive RNA-based *NF1* testing, we designed the **customized and optimized NGS *NF1*-component** of the assay to comprise **all regions** encountered through analysis of >15,000 unrelated individuals including >8,100 *NF1*-variant-positive individuals carrying 1 out of >3,100 different unique ***NF1*** variants identified in the UAB MGL cohort. Included in the NGS assay are the regions covering >65 different deep intronic splice variants (which reside beyond the +/-50 intronic base pairs that flank all exons). Validation of the full panel included, besides substitutions (missense, nonsense, splice variants), the most challenging variants such as insertions/deletions/duplications of 1-112bp (~25% of the UAB NF1 cohort) and one-to-multiple exon deletions/duplications (~2.8% of the UAB NF1 cohort). The analytical sensitivity of our NGS testing approach was 100% for substitutions as well as insertion/deletions up to 112bp. The panel has been validated for the detection of germline (heterozygous) single-exon deletions/duplications as well as multi-exon deletions/duplications. Single exon deletions/duplications are present in ~0.45% of *NF1*-positive patients from the UAB cohort with 9% of these individuals being mosaic (~0.045% of all in the UAB *NF1*-positive cohort). Detection of Alu/LINE insertions, identified in 0.25% of patients from the UAB *NF1*-positive cohort, has not yet been validated using the current NGS approach.

With the **largest dataset of *NF1* genotypes matched with phenotypes,** any genotype-phenotype correlations identified will be reported in real time.

For **novel *NF1* variants of unknown significance**, we offer free of charge targeted RNA-based testing to assess the effect of the variant on splicing and enhance the correct classification/ interpretation.

Relevant family members of a proband with any (novel or previously identified) variant of unknown significance are offered free of charge targeted analysis as long as accurate phenotypic data are provided by a health care professional to enhance the interpretation. There is no limitation to the number of relatives that can be tested free of charge.

Mosaicism is often present in sporadic patients with an ***NF1* microdeletion** and has important repercussions for counseling (Kehrer-Sawatzki H, Mautner VF, Cooper DN. 2017). Evaluation by **FISH analysis on 200 interphase chromosomes** can be offered in such cases.

NF1/SPRED1 by NGS

Candidates for this test: patients with multiple CALMs with or without skinfold freckling and no other typical *NF1*-associated features (Lisch nodules, bone abnormalities, neurofibromas, optic pathway gliomas).

The **DNA-based *NF1/SPRED1*-only by NGS** involves **sequencing** as well as **deletion/duplication analysis** of the entire coding *NF1* region plus the alternatively spliced exons 9br, 23a and 48a (60 exons total), as well as sequencing and **deletion/duplication analysis** for *SPRED1*. The test uses an extensively customized and optimized set of Agilent HaloPlex capture probes, followed by sequencing of overlapping amplicons within the regions of interest using 300bp paired-end Illumina sequencing chemistry. Each coding exon plus ~50bp of flanking intronic sequence are simultaneously sequenced. 5' and 3' untranslated sequences are not included. The **average coverage is >1600x with >98% of the *NF1* coding region ≥350x and 99% ≥200x, allowing detection of very low level mosaicism, down to 3-5% variant allele fraction respectively (regions covered by ≥350x and ≥200x respectively) with 95% confidence**. Variant and copy number calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels, and frameshifts caused by deletion/ duplication up to 112bp.

Expanded *NF1*-RASopathy by NGS

Candidates for this test: patients with clinical features suggestive of either NS, NSML, CFC, NF1, Legius syndrome or Noonan-like syndrome; patients with a clinical diagnosis of any of these syndromes who previously tested negative in a subset of the genes included in this panel; patients with a diagnosis of Costello syndrome but no *HRAS* variant was previously identified.

The **Expanded *NF1*-Rasopathy panel by NGS** test involves the simultaneous sequencing of **18 genes**: *NF1, SPRED1, LZTR1, PTPN11, PPP1CB, BRAF, CBL, HRAS, KRAS, NRAS, MAP2K1, MAP2K2, RAF1, RIT1, RASA2, SHOC2, SOS1* and *SOS2*. The test uses the same approach as detailed on page 7 (see: *NF1*-only by NGS). The **average coverage of the Expanded *NF1*-Rasopathy panel by NGS is >1600x with >98% of the coding regions ≥350x and 99% ≥200X, allowing detection of very low level mosaicism, down to 3-5% variant allelle fraction respectively (regions covered by ≥350x and ≥200x respectively) with 95% confidence**. The minimum coverage for any additional areas is >30x. Variant and copy number calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels and frameshifts caused by a deletion or duplication up to 112bp. **Deletion/duplication analysis** for *NF1, SPRED1,* and *LZTR1* is included in this test, as such variants are a part of the mutation spectrum for these conditions. Deletion/duplication analysis for the other 15 genes on this panel is not offered as current empirical and biological evidence is not sufficient to allow the conclusion that an altered copy number of these genes is a mechanism critical for the phenotype associated with the Rasopathies.

Non-*NF1* RASopathy NGS Panel

Candidates for this test: patients with clinical features suggestive of either NS, NSML, CFC, Legius syndrome or Noonan-like syndrome, and/or with no variant previously found by comprehensive RNA-based *NF1*+/-*SPRED1* testing.

The **non-*NF1* Rasopathy by NGS** involves the simultaneous **sequencing** of **17 genes:** *SPRED1, LZTR1, PTPN11, PPP1CB, BRAF, CBL, HRAS, KRAS, NRAS, MAP2K1, MAP2K2, RAF1, RIT1, RASA2, SHOC2, SOS1 and SOS2*. The test uses the same approach as detailed on page 7 (see: *NF1*-only by NGS). The **average coverage of the Non-*NF1* RASopathy NGS panel is >1600x with >98% of the coding regions ≥350x and 99% ≥200x, allowing detection of very low level mosaicism, down to 3-5% variant allele fraction (regions covered by ≥350x and ≥200x respectively) with 95% confidence.** The minimum coverage for any additional areas is >30x. Variant and copy number calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels and frameshifts caused by a deletion or duplication up to 112bp. **Deletion/duplication analysis** for *SPRED1* and *LZTR1* is included in this test, as such variants are a part of the mutation spectrum for this gene. Deletion/duplication analysis for the other 15 genes on this panel is not offered as current empirical and biological evidence is not sufficient to allow the conclusion that an altered copy number of these genes is a mechanism critical for the phenotype associated with the Rasopathies.

Costello syndrome *HRAS*-only by NGS

Candidates for this test: patients with key CS features including coarse facial features, severe feeding difficulty, mild to moderate intellectual disability, relative macrocephaly and short stature, high incidence of cardiac abnormalities and malignancy. Differentiation of CS from other RASopathies, particularly CFC may be difficult especially early in life.

The **DNA-based *HRAS*-only by NGS** involves sequencing of the entire coding *HRAS* regions. The test uses the same approach as detailed on page 7 (see: *NF1*-only by NGS). **The average coverage is >1100x with 100% of the coding region covered at ≥350x.** This allows for detection of very low level mosaicism by sequencing (as low as 3% of the alleles in 100% of the coding region with >350x). Variant calls are made using a unique bioinformatics pipeline.

Capillary Malformation/Parkes Weber *RASA1* and *EPHB4* by NGS

Candidates for this test: patients with features suggestive for capillary malformation-arteriovenous malformation or Parkes Weber syndrome.

The **DNA-based *RASA1* and *EPHB4* by NGS** involves sequencing of *RASA1* and *EPBH4* as well as **deletion/duplication analysis** of the entire coding *RASA1* region using MLPA. The test uses the same approach as detailed on page 7 (see: *NF1*-only by NGS). **The average coverage is >1500x with 93% of the coding region covered at ≥350x and 97% at ≥200x.** This allows for detection of very low level mosaicism by sequencing (as low as 3-5% of the alleles). Variant calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels and frameshifts caused by a deletion or duplication up to 112bp.

GNAS-only by NGS

Candidates for this test: Patients with abnormal CAL spots (frequently large and with irregular borders); clear polyostotic fibrous dysplasia, which may present as fractures, uneven bone growth, or deformity; and/or precocious puberty or other endocrine problems.

The **DNA-based *GNAS*-only by NGS** involves NextGen sequencing of **exons 8 and 9** of the *GNAS* gene with specific high coverage at the codons p.Arg201 and p.Gln227. The test uses an extensively customized and optimized set of Agilent HaloPlex capture probes, followed by sequencing of overlapping amplicons within the regions of interest using 300bp paired-end Illumina sequencing chemistry. Each coding exon plus ~50bp of flanking intronic sequence are simultaneously sequenced. 5' and 3' untranslated sequences are not included. The **average coverage of the GNAS-only by NGS for exons 8 and 9 is >1600x**, allowing detection of very low level mosaicism, down to 3% variant allele fraction with 95% confidence.

NF2-only by NGS

Candidates for this test: patients with bilateral vestibular schwannomas with or without other typical NF2-associated features (congenital cataracts, ependymoma, facial weakness, etc).

The **DNA-based *NF2*-only by NGS** involves **sequencing** as well as **deletion/duplication analysis** of the entire coding *NF2* regions using MLPA. The test uses the same approach as detailed on page 7 (see: *NF1*-only by NGS). The **average coverage is >1600x with 100% of the coding region covered at ≥350x.** This allows for detection of very low level mosaicism by sequencing (as low as 3% of the alleles in 100% of the coding region with >95% confidence)**.** Variant calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels and frameshifts caused by a deletion or duplication up to 112bp. Deletion/duplication analysis for *NF2* using MLPA is included in this test, as such variants are a part of the mutation spectrum for this condition.

Schwannomatosis/Multiple Schwannomas Panel by NGS

Candidates for this test: patients with multiple schwannomas with or without vestibular schwannomas. This testing approach is developed to diagnose mosaic NF2, classic NF2, schwannomatosis, and mosaic schwannomatosis.

The **Schwannomatosis/Multiple Schwannomas NGS panel** involves the simultaneous sequencing of **3 genes**: *NF2, SMARCB1*, and *LZTR1*. The test uses the same approach as detailed on page 7 (see: *NF1*-only by NGS). The **average coverage is >1600x with 100% of the coding region covered at ≥350x**. This allows for detection of very low level mosaicism by sequencing (as low as 3% of the alleles in 100% of the coding region with >95% confidence). Variant calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels and frameshifts caused by a deletion or duplication up to 112bp. Deletion/duplication analysis for *NF2, SMARCB1, and LZTR1* using MLPA is included in this test, as such variants are a part of the mutation spectrum for these conditions. .

Meningiomatosis/Multiple Meningiomas Panel by NGS

Candidates for this test: patients with clinical features suggestive of meningiomatosis or NF2 with the inclusion of meningiomas within their phenotype.

The **Meningiomatosis/Multiple Meningiomas panel by NGS** involves the simultaneous sequencing of 4 genes: *NF2, SMARCB1, SMARCE1,* and *SUFU*. The test uses the same approach as detailed on page 7 (see: *NF1*-only by NGS). The **average coverage is >1500x with >99.9% of the coding region ≥350x and 100% ≥200x.** This allows for detection of very low level mosaicism by sequencing (as low as 3% of the alleles in 100% of the coding region with >95% confidence). Variant calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels and frameshifts caused by a deletion or duplication up to 112bp. Deletion/duplication analysis for *NF2 and SMARCB1* using MLPA is included in this test, as such variants are a part of the mutation spectrum for these conditions. Deletion/duplication analysis for the other genes on this panel is not offered as current empirical and biological evidence is not sufficient to allow the conclusion that an altered copy number of these genes is a mechanism critical for the phenotype associated with these condivtions.

Peripheral Nerve Sheath Tumor Panel by NGS

Candidates for this test: patients presenting with both neurofibromas and schwannomas **or** peripheral nerve sheath tumors with mixed cellularity, with minimal additional findings and not meeting diagnostic criteria for any specific condition.

The **Peripheral Nerve Sheath Tumor Panel by NGS** involves the simultaneous sequencing of **6 genes**: *NF1, NF2, KRAS, LZTR1, PTPN11,* and *SMARCB1*. The test uses the same approach as detailed on page 7 (see: *NF1*-only by NGS). The **average coverage is >1600x with >98% of the coding region covered at ≥350x and 99% covered at ≥200x**. The minimum coverage for any additional areas is >30x. This allows for detection of very low level mosaicism by sequencing (as low as 3% of the alleles in >99.9% of the coding region with coverage >350x). Variant calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels and frameshifts caused by a deletion or duplication up to 112bp. Deletion/duplication analysis for *NF1, NF2, SMARCB1, and LZTR1* using MLPA is included in this test, as such variants are a part of the mutation spectrum for these conditions. Deletion/duplication analysis for *PTPN11* and *KRAS* not offered as current empirical and biological evidence is not sufficient to allow the conclusion that an altered copy number of *PTPN11* and *KRAS* is a mechanism critical for the phenotype associated with these conditions.

Rhabdoid Tumor Predisposition syndrome by NGS

Candidates for this test: patients with rhabdoid tumors with or without confirmed *SMARCB1*-loss identified in the tumors by immunohistochemistry staining.

The **DNA-based Rhabdoid Tumor Predisposition syndrome by NGS** test involves **sequencing** of *SMARCB1* [and *SMARCA4* when testing on blood, saliva, or extracted DNA] as well as **deletion/duplication analysis** of the entire coding *SMARCB1* regions using MLPA. The test uses the same approach as detailed on page 7 (see: *NF1*-only by NGS). The **average coverage is >1100x with 91% of the coding regions ≥350x and 95% ≥200x**. The minimum coverage for any additional areas is >30x. Variant calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels, and frameshifts caused by deletion/ duplication up to 112bp.

Tuberous Sclerosis Complex Panel by NGS

Candidates for this test: patients with clinical features suggestive of Tuberous Sclerosis Complex.

The **Tuberous Sclerosis Complex panel by NGS** involves the simultaneous sequencing of 2 genes: *TSC1 and TSC2*. The test uses the same approach as detailed on page 7 (see: *NF1*-only by NGS). The **average coverage is >1600x with >99% of the coding region covered at ≥350x and >99% ≥200x**. The minimum coverage for any additional areas is >30x. This allows for detection of very low level mosaicism by sequencing (as low as 3% of the alleles in >99.5% of the coding region with coverage >350x with 95% confidence). Variant calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels and frameshifts caused by a deletion or duplication up to 112bp. Deletion/duplication analysis for *TSC1 and TSC2* using MLPA is included in this test, as such variants are a part of the mutation spectrum for these conditions.

❖*RNA-based Testing via Sanger Sequencing*❖

TEST OPTIONS

- RNA-based *NF1*/DNA-based *SPRED1* testing on blood
- RNA-based *NF1*-only testing on blood
- RNA-based *NF1*/DNA-based *SPRED1* testing on affected tissues

SPECIMEN TYPES

- Fresh EDTA blood sample, to arrive in the lab <60-72 hours after blood draw
- Biopsies of café-au-lait macules (CALMs) and/or neurofibromas

RNA-based *NF1* and DNA-based *SPRED1* testing on blood

Candidates for this test: patients who need the most sensitive and specific test with the fastest turnaround time.

The **RNA-based *NF1* and gDNA-based *SPRED1* testing** on blood requires **a fresh EDTA blood** sample, to arrive in the lab <60-70 hours after blood draw. **DNA** is extracted and, in addition, a short term phytohemagglutinin-stimulated lymphocyte culture is initiated and used as starting material to extract **RNA**. The complete *NF1* coding region is analyzed by a cascade of complementary variant detection techniques, including RT-PCR, direct sequencing of cDNA fragments, microsatellite marker analysis, copy number analysis by MLPA and interphase FISH (if needed), enabling identification of the variant in ~95% of non-founder patients fulfilling the NIH diagnostic criteria (Messiaen et al, Hum Mutat.2000;15(6);541-55; Messiaen and Wimmer, Hum Genet.2008;(16);63-7).

RNA-based *NF1* testing allows finding deep intronic splice variants through their observed effect on splicing. During the >15 years we have offered comprehensive RNA-based *NF1* testing on blood, we have identified >65 different deep intronic splice variants: they account for 2.5% of all pathogenic mutations identified in the NF1 UAB cohort. Please note, however, that all known deep intronic splice variants have been incorporated in the *customized* UAB NGS assay described above.

In addition, all coding exons and flanking intronic sequences of the *SPRED1* gene are analyzed by bidirectional sequencing and deletion/duplication analysis is performed using MLPA.

RNA-based *NF1* testing on blood

Candidates for this test: 1) patients who need the most sensitive and specific test with the fastest TAT (e.g. patients with an ongoing pregnancy); and 2) non-founder patients a) with clear-cut clinically documented classic NF1, b) from a clinically documented multi-generation (minimum 3 generations) family, c) who tested negative by the MGL ***NF1*-only by NGS** assay and d) in whom a translocation has been excluded by cytogenetic analysis. The latter patients will receive free of charge reflex **RNA-based *NF1* testing**, which should allow to detect a possible deep intronic splice variant not previously identified in the UAB cohort and not reported elsewhere, or a possible Alu/LINE insertion or other exotic complex variant. Based on current data, such variants account conservatively for <0.25% of familial cases.

NF1 testing is performed as described in the previous section.

RNA-based *NF1/SPRED1* testing on cultured cells from affected tissues

Candidates for this test: Individuals suspected to have segmental NF1, with symptoms restricted to a defined area of the body; sporadic patients who have (mild) non-localized symptoms of NF1 but in whom no *NF1* variant was identified in the blood lymphocytes and may have disease due to a postzygotic variant; reflex testing for familial or sporadic patients with a first hit variant refractory to detection by RNA- or DNA/NGS assay.

The **RNA-based *NF1/SPRED1* testing on cultured cells from affected tissues** is offered starting from **biopsies** of café-au-lait macules (CALM) and/or neurofibromas. Melanocytes cultured from CALMs and Schwann cells cultured from neurofibromas are the starting material for the cascade of complementary variant detection techniques described in the previous section on "RNA-based *NF1* testing on blood" (Maertens et al, 2007, De Schepper et al, 2007). In addition, for patients with only pigmentary features (CALMs w/wo skinfold freckling but no neurofibromas), and no *NF1* variants found in the melanocytes (no first or second hit variants), the *SPRED1* gene is analysed (sequencing and deletion/duplication analysis), as these patients may have mosaic Legius syndrome. As a result of this test, if features are *NF1* or *SPRED1*-related, a common first hit is identified in both biopsies and a different second hit is identified in every anatomically different biopsy evaluated. If no variants are identified despite full analysis on 2 biopsies with successful cultures, (mosaic) NF1/Legius syndrome is very unlikely (<0.1%).

We require a minimum of 2 biopsies, ideally 3, (3-5 mm punch biopsies) from anatomically different affected locations. Please contact us at **medgenomics@uabmc.edu** or **205-934-5562** to set up a time to discuss your patient **prior** to taking biopsies from your patient, so we can provide individualized advice and ship out appropriate collection/transport media and forms prior to the procedure.

Please contact us at medgenomics@uabmc.edu or 205-934-5562 if you have questions or want to discuss genetic testing needs for your patients. We will be happy to help.

Variant Confirmation Policy

Our Next-Generation Sequencing method is designed for deep coverage (average ≥1600X). In the very rare event where the coverage at a specific site may fall below 30X, Sanger sequencing is performed to cover any such low coverage region for all samples without a clear-cut or likely pathogenic variant. Sanger sequencing is performed as confirmatory testing for all pathogenic variants, likely pathogenic variants, or variants of uncertain significance which are identified by NGS analysis when present with a VAF of ≤40%. In addition, confirmatory Sanger sequencing is also performed for all pathogenic, likely pathogenic, or variants of uncertain significance which are identified by NGS analysis to be present with VAF of >40% if i) the Q-score is <30, or ii) if the variant is present in only one amplicon, or iii) if present in just one direction, and/or iv) if the variant is present in a region with coverage ≤200X. Sanger sequencing is also performed for confirmation of all indel variants, as Sanger sequencing will allow to accurately determine the variant nomenclature according to HGVS variant nomenclature recommendations. Additional Sanger sequencing and High Resolution Melting Curve analysis are performed for all pathogenic, likely pathogenic, or variants of uncertain significance when the VAF is <10%, and/or when confirmation by routine PCR is not conclusive. Sanger sequencing is not performed for confirmation of known neutral polymorphisms and these are not included in the patient report. Repeated sample library construction and NGS analysis may be performed to verify the very low level of variants, if necessary. Sanger sequencing is performed using dye-terminator chemistry on an ABI PRISM 3130 or 3730 capillary sequencer (Applied Biosystems®).

References

- Aavikko M, Li SP, Saarinen S, Alhopuro P, Kaasinen E, Morgunova E, Li Y, Vesanen K, Smith MJ, Evans DG, Pöyhönen M, Kiuru A, Auvinen A, Aaltonen LA, Taipale J, Vahteristo P. Loss of SUFU function in familial multiple meningioma. *Am J Hum Genet. 2012 Sep 7;91(3):520-6.*
- Bayrak-Toydemir P, Stevenson D. Capillary Malformation-Arteriovenous Malformation Syndrome. 2011 Feb 22 [Updated 2019 Sep 12]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK52764/
- Bertola DR, Pereira AC, Brasil AC, Suzuki L, Leite C, Falzoni R, Tannuri U, Poplawski AB, Janowski KM, Kim CA, Messiaen LM. Multiple, diffuse schwannomas in a RASopathy phenotype patient with germline KRAS mutation: a causal relationship? *Clin Genet. 2012 Jun;81(6):595-7.*
- Boyce AM, Florenzano P, de Castro LF, et al. Fibrous Dysplasia/McCune-Albright Syndrome. 2015 Feb 26 [Updated 2019 Jun 27]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK274564/
- Brems H, Chmara M, Sahbatou M, Denayer E, Taniguchi K, Kato R, Somers R, Messiaen L, De Schepper S, Fryns J-P, Cools J, Marynen P, Thomas G, Yoshimura A, Legius E. Germline loss-of function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype. *Nat Genet 39: 1120-1126, 2007.*
- Conboy E, Dhamija R, Wang M, Xie J, Dyck PJ, Bridges AG, Spinner RJ, Clayton AC, Watson RE, Messiaen L, Babovic-Vuksanovic D. Paraspinal neurofibromas and hypertrophic neuropathy in Noonan syndrome with multiple lentigines. *J Med Genet. 2016: 53(2):123-6.*
- De Schepper S, Maertens O, Callens T, Naeyaert JM, Lambert J, Messiaen L. (2008) Somatic mutation analysis in NF1 café au lait spots reveals two NF1 hits in the melanocytes. *J Invest Dermat,128(4): 1050-3.*
- Eerola I, Boon LM, Mulliken JB, Burrows PE, Dompmartin A, Watanabe S, Vanwick R, Vikkula M. Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. *Am J Hum Genet. 2003 Dec;73(6):1240-9.*
- Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. *J Med Genet. 2002 May;39(5):311-4.*
- Evans DG, Ramsden RT, Shenton A, Gokhale C, Bowers NL, Huson SM, Pichert G, Wallace A. Mosaicism in neurofibromatosis type 2: an update of risk based on uni/bilaterality of vestibular schwannoma at presentation and sensitive mutation analysis including multiple ligation-dependent probe amplification. *J Medical Genet. 2007 Jul 1;44(7):424-8.*
- Gripp K, Aldinger K, Bennett J, Baker L, Tusi J, Powell-Hamilton N, Stabley D, Sol-Church K, Timms A, Dobyns W B A Novel Rasopathy Caused by Recurrent De Novo Missense Mutations in *PPP1CB* Closely Resembles Noonan Syndrome with Loose Anagen Hair. *Am J Med Genet A.* 2016 September; 170(9): 2237-2247.
- Hulsebos TJ, Plomp AS, Wolterman RA, Robanus-Maanadaq EC, Baas F, Wesseling P. Germline mutation of INI1/SMARCB1 in familial schwannomatosis. *Am J Hum Genet. 2007 Apr;80(4):805-10.*

References

- Kehrer-Sawatzki H, Mautner VF, Cooper DN. Emerging genotype-phenotype relationships in patients with large NF1 deletions. Hum Genet. 2017;136(4):349–376.
- Korf B, Ahmadian R, Allanson J, Aoki Y, Baller A, Burkitt-Wright E, Denger B, Elgersma Y, Gelb B, Gripp K, Kerr B, Kontaridis M, Lazaro C, Linarid C, Lozano R, MacRae CA, Messiaen L, Mulero-Navarro S, Neel B, Plotkin S, Rauen KA, Roberts A, Silva AJ, Sittampalam S, Zhang C and Schoyer L. The third international meeting on genetic disorders in the RAS/MAPK pathway: towards a therapeutic approach. *Am J Med Genet. A 2015: 167(8): 1741-1746.*
- Maertens O, De Schepper S, Vandesompele J, Brems H, Heyns I, Janssens S, Speleman F, Legius E, Messiaen L (2007) Molecular dissection of isolated disease features in mosaic neurofibromatosis type 1. *Am J Hum Genet 81(2): 243-251.*
- Magdalena Koczkowska, Yunjia Chen, Tom Callens, Sherrell Johnson, Alicia Gomes, Meng-Chang Hsiao, Angela Sharp, Meena Balasubramanian, Christopher P. Barnett, Troy Becker, Shay Ben-Shachar, Debora R. Bertola, Jaishri Blakeley, Emma M.M. Burkitt-Wright,Chelsea Chambers, Melissa Crenshaw, Karin S.G. Cunha, Maria D. D'Agostino, Karin Dahan, Alessandro De Luca, Anne Destrée, Radhika Dhamija, Marica Eoli, D. Gareth R. Evans, Patricia Galvin-Parton, Jaya George-Abraham, Karen W. Gripp, Jose Guevara-Campos, Neil Hanchard, Concepcion Hernández-Chico, LaDonna Immken, Sandra Janssens, Kristi Jones, Beth A. Keena, Aaina Kochhar, Jan Liebelt, Arelis Martir-Negron, Maurice J. Mahoney, Isabelle Maystadt, Carey McDougall, Nancy Mendelsohn, David Miller, Geert Mortier, John Pappas, Scott Plotkin, Dinel Pond, Kenneth Rosenbaum, Daniel S. Roy, Karol Rubin, Laura Russell, Lane S. Rutledge, Veronica Saletti, Rhonda Schonberg, Allison Schreiber, Meredith Seidel, David Stockton, Eva Trevisson, Nicole Ulrich, Meena Upadhyaya, Rick van Minkelen, Heleen Verhelst, Margaret R. Wallace, Elaine Zackai, Jonathan Zonana, Vickie Zurcher, Kathleen Claes, Yolanda Martin, Bruce R. Korf, Eric Legius,. Ludwine Messiaen. Genotype-phenotype correlation in NF1 patients: evidence for a more severe phenotype associated with missense mutations affecting NF1 codons 844-848. Am J. Hum. Genet. 2018, 102(1):69-87. PubMed PMID: 29298338
- Messiaen LM, Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N, Speleman F, De Paepe A (2000) Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. *Hum Mutat 15(6):541-55.*
- Messiaen LM and Wimmer K (2008) NF1 Mutational Spectrum, in Kaufmann D (ed): Neurofibromatoses. *Monogr Hum Genet. Basel, Karger, Vol 16:63-7.*
- Messiaen L, Yao S, Brems H, Callens T, Sathienkijkanchai A, Denayer E, Spencer E, Arn P, Babovic-Vuksanovic D, Bay C, Bobele G, Cohen BH, Escobar L, Eunpu D, Grebe T, Greenstein R, Hachen R, Irons M, Kronn D, Lemire E, Leppig K, Lim C, McDonald M, Narayanan V, Pearn A, Pedersen R, Powell B, Shapiro LR, Skidmore D, Tegay D, Thiese H, Zackai EH, Vijzelaar R, Taniguchi K, Ayada T, Okamoto F, Yoshimura A, Parret A, Korf B, Legius E. Clinical and Mutational Spectrum of Neurofibromatosis type 1-like syndrome. *JAMA 2009: 302(19): 2111-8.*
- Northrup H, Krueger DA; International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 Iinternational Tuberous Sclerosis Complex Consensus Conference. *Pediatr Neurol. 2013 Oct;49(4):243-54.*
- Nyström AM, Ekvall S, Berglund E, Björkqvist M, Braathen G, Duchen K, Enell H, Holmberg E, Holmlund U, Olsson-Engman M, Annerén G, Bondeson ML. – Noonan and cardio-facio-cutaneous syndromes: two clinically and genetically overlapping disorders. *J Med Genet. 2008: 45(8):500-6.*
- Piotrowski A, Xie J, Liu YF, Poplawski AB, Gomes AR, Madanecki P, Fu C, Crowley MR, Crossman DK, Armstrong L, Babovic-Vuksanovic D, Bergner A, Blakeley JO, Blumenthal AL, Daniels MS, Feit H, Gardner K, Hurst S, Kobelka C, Lee C, Nagy R, Rauen KA, Slopis JM, Suwannarat P, Westman JA, Zanko A, Korf BR, Messiaen LM. Germline loss-of-function mutations in LZTR1 predispose to an inherited disorder of multiple schwannomas. *Nat Genet. 2014 Feb;46(2):182-7.*
- Sancak O, Nellist M, Goedbloed M, Elfferich P, Maat-Kievit A, Zonnenberg B, Verhoef S, Halley D, van den Ouweland A. Mutational analysis of the TSC1 and TSC2 genes in a diagnostic setting: genotype--phenotype correlations and comparison of diagnostic DNA techniques in Tuberous Sclerosis Complex. Eur J Hum Genet. 2005 Jun; 13(6):731-41.
- Rauen K. The Rasopathies. *Annu Rev. Genom. Hum. Genet. 2013: 14: 355-369.*

References

- Rauen K, Huson S, Burkitt-Wright E, Evans DG, Farschtschi S, Ferner RE, Gutmann D, Hanemann O, Kerr B, Legius E, Parada L, Patton M, Peltonen J, Ratner N, Riccardi V, van der Vaart T, Vikkula M, Viskochil D, Zenker M, Updahyaya M. Recent Developments in Neurofibromatosis and RASopathies: Management, Diagnosis and Current and Future Therapeutic Avenues. *Am. J. Med Genet A 2015: 167(1): 1-10.*
- Rojnueangnit K, Xie J, Gomes A, Sharp A, Callens T, Chen Y, Liu Y, Cochran M, Abbott MA, Atkin J, Babovic-Vuksanovic D, Barnett CP, Crenshaw M, Bartholomew DW, Basel L, Bellus G, Ben-Shachar S, Bialer MG, Bick D, Blumberg B, Cortes F, David KL, Destree A, Duat-Rodriguez A, Earl D, Escobar L, Eswara M, Ezquieta B, Frayling IM, Frydman M, Gardner K, Gripp KW, Hernández-Chico C, Heyrman K, Ibrahim J, Janssens S, Keena BA, Llano-Rivas I, Leppig K, McDonald M, Misra VK, Mulbury J, Narayanan V, Orenstein N, Galvin-Parton P, Pedro H, Pivnick EK, Powell CM, Randolph L, Raskin S, Rosell J, Rubin K, Seashore M, Schaaf CP, Scheuerle A, Schultz M, Schorry E, Schnur R, Siqveland E, Tkachuk A, Tonsgard J, Upadhyaya M, Verma IC, Wallace S, Williams C, Zackai E, Zonana J, Lazaro C, Claes K, Korf B, Martin Y, Legius E, Messiaen L. High incidence of Noonan Syndrome Features including Short Stature and Pulmonic Stenosis in Patients carrying NF1 Missense Mutations affecting p.Arg1809: Genotype-Phenotype Correlation. *Hum Mutat. 2015: 36(11):1052-63.*
- Sancak O, Nellist M, Goedbloed M, Elfferich P, Wouters C, Maat-Kievit A, Zonnenberg B, Verhoef S, Halley D, van den Ouweland A. Mutational analysis of the TSC1 and TSC2 genes in a diagnostic setting: genotype--phenotype correlations and comparison of diagnostic DNA techniques in Tuberous Sclerosis Complex. *Eur J Hum Genet. 2005 Jun;13(6):731-41.*
- Serrano C, Simonetti S, Hernandez-Losa J, Valverde C, Carrato C, Baque S, Orellana R, Somoza R, Moline T, Carles J, Huquet P, Romagosa C, Ramon y Cajal S. BRAF V600E and KRAS G12S mutations in peripheral nerve sheath tumors. *Histopathology. 2013 Feb;62(3):499-504.*
- Sévenet N, Lellouch-Tubiana A, Schofield D, Hoang-Xuan K, Gessler M, Birnbaum D, Jeanpierre C, Jouvet A, Delattre O. Spectrum of hSNF5/INI1 somatic mutations in human cancer and genotype-phenotype correlations. *Hum Mol Genet. 1999 Dec;8(13):2359-68*
- Smith MJ, Higgs JE, Bowers NL, Halliday D, Paterson J, Gillespie J, Huson SM, Freeman SR, Lloyd S, Rutherford SA, King AT, Wallace AJ, Ramsden RT, Evans DG. Cranial meningiomas in 411 neurofibromatosis type 2 (NF2) patients with proven gene mutations: clear positional effect of mutations, but absence of female severity effect on age at onset. *J Med Genet. 2011 Apr;48(4):261-5.*
- Smith MJ, Wallace AJ, Bennett C, Hasselblatt M, Elert-Dobkowska E, Evans LT, Hickey WF, van Hoff J, Bauer D, Lee A, Hevner RF, Beetz C, du Plessis D, Kilday JP, Newman WG, Evans DG. Germline SMARCE1 mutations predispose to both spinal and cranial clear cell meningiomas. *J Pathol. 2014 Dec;234(4):436-40.*
- van den Munckhof P, Christiaans I, Kenter SB, Baas F, Hulsebos TJ. Germline SMARCB1 mutation predisposes to multiple meningiomas and schwannomas with preferential location of cranial meningiomas at the falx cerebri. *Neurogenetics. 2012 Feb;13(1):1-7.*

Notes

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UAB MEDICAL GENOMICS LABORATORY

The Medical Genomics Laboratory in the Department of Genetics at UAB, directed by Ludwine Messiaen, Ph.D, FACMG, is an academic non-profit clinical lab, CLIA and CAP certified. Our dedicated and experienced staff takes pride in serving the patients and referring physicians the best we can.

We work closely with the UAB Neurofibromatosis clinic led by neurologists and geneticists **Dr. Bruce Korf**, Wayne H. and Sara Crews Finley endowed Chair in Medical Genetics at UAB.

The clinic serves patients and their families dealing with the lifelong medical, psychological and social implications of the various forms of neurofibromatoses and RASopathies.

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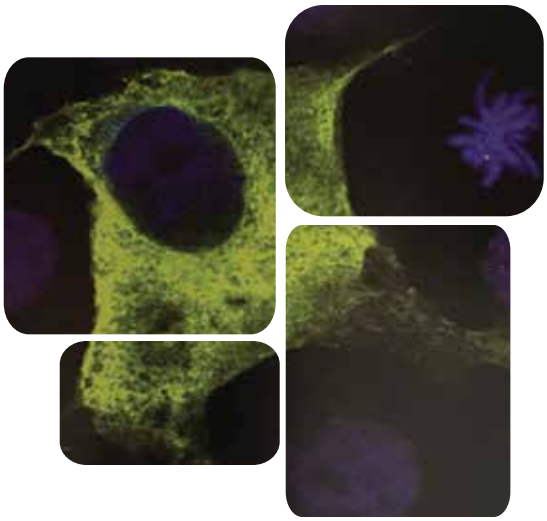

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