# Primer on the Neurofibromatoses for ASGCT Career Development Award

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by Peggy Wallace, PhD, University of Florida,
on behalf of
the Children's Tumor Foundation: Ending NF Through Research

Conflicts of interest: none.

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Outline

Neurofibromatosis type 1 (NF1)
- clinical – greatest morbidity from tumors (neurofibromas)
- gene/protein
- mechanisms/cell types

NF2-related Schwannomatosis (NF2-SWN) [previously called NF2]
- clinical - greatest morbidity from tumors (schwannomas, meningiomas, ependymomas)
- gene/protein
- mechanisms/cell types

Non-NF2 Schwannomatosis (SWN)
- clinical aspects – greatest morbidity from pain, not always caused by schwannoma
- gene/protein
- mechanisms/cell types

Schwann cells (SC) in normal, healthy peripheral nerve: fully differentiated, wrap axons, produce myelin, quiescent. In response to stimuli (e.g tissue injury), SC de-differentiate and proliferate. Once homeostasis is regained, SC differentiate again.

Neurofibromatoses: all have inappropriate proliferation of SC to form benign tumors.

# Why Gene Therapy for Neurofibromatoses?

Together, they may affect as many as 1/2500 individuals worldwide.

No effective therapies except one FDA approved drug to stop/shrinkinoperable plexiform tumors in children with NF1: selumetinib – a MEK inhibitor, Koselugo brand name, effective in 70% of cases. Has some side effects. Tumor may start growing again after ceasing treatment.

Treatments are symptomatic, and often ineffective. For example, if surgery does not completely excise a tumor, it could grow back; however surgery has high risk for nerve damage. Most individuals with these conditions have significant reductions in quality of life.

Investing in multiple therapeutic approaches for each condition because one alone will likely not be effective for 100% of patients.

# NF1 current diagnostic criteria

A. If *neither* parent has NF1, the individual must have ≥ *2 of these 7 criteria* for diagnosis:

- 6 or more café-au-lait macules (CALs) at least 5 mm in greatest diameter in prepubertal individuals, and at least 15 mm in greatest diameter in postpubertal individuals
- Freckling of the axial or inguinal region
- Two or more neurofibromas of any type, or one plexiform neurofibroma
- Optic pathway glioma
- Two or more iris Lisch nodules identified by slit lamp examination, or two or more choroidal abnormalities (CAs)—defined as bright, patchy nodules imaged by optical coherence tomography (OCT)/near-infrared reflectance (NIR) imaging
- Distinctive osseous lesion such as sphenoid dysplasia, anterolateral bowing of the tibia, or pseudoarthrosis of a long bone
- heterozygous pathogenic *NF1* gene variant (the only gene causing NF1), with variant allele fraction of ~50% in apparently normal tissue such as white blood cells

B. A child of a parent who meets the diagnostic criteria specified in A merits a diagnosis of NF1 if one or more of the criteria in A are present.

**Neurofibromas** - peripheral nerve endings in skin, anytime in life. May be itchy and/or painful. Two events involved: independent somatic mutation of non-germline *NF1* allele (Knudson two-hit mechanism) in Schwann cells or their precursors, and microenvironment signals. No consistent additional genetic alterations in typical neurofibromas. Neurofibromas that cause functional deficits or disfigurement result in psychosocial burden as well. *Most NF1 features are 2-hit phenomena*.

## Cutaneous (usually don't affect function directly)

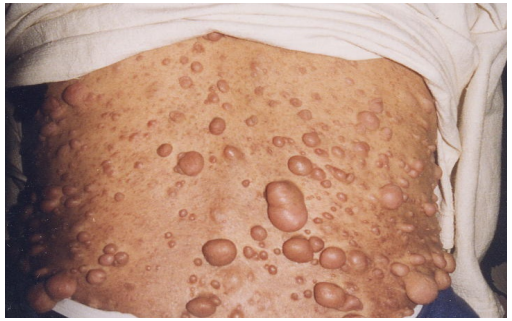
- Depth in skin can vary (virtually flat to pedunculated)
- Arise at/near end of nerve twigs in skin, can be itchy or painful
- Slow growth rate (can vary), size usually less than 10 mm diameter
- Usually begin at/after puberty, do not transform

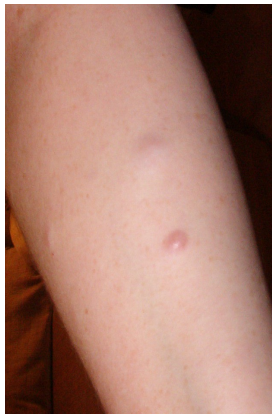
## Plexiform (may have severe functional effect or result in fatality)

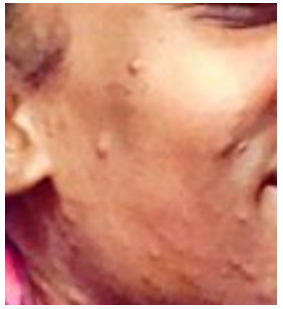
- Intraneural - can grow outward or up/down the nerve, impinging on tissues, often painful.
- Slow growth rate (can vary), no upper size limit.
- Congenital, some evident at birth or early childhood, others might not be detected for years.
- Highly vascular.
- Growth most rapid in childhood but cannot be predicted.
- 10-15% risk of transformation to malignant peripheral nerve sheath tumor (**MPNST**), a soft-tissue sarcoma with poor survival (most occurring in age range 15 – 40 yr). **Atypical neurofibroma** (ANNUBP) has acquired loss of *CDK2A/B,* and is likely an intermediate to MPNST. Additional genomic changes leading to malignancy usually include loss of PRC2 activity.

# Neurofibromas

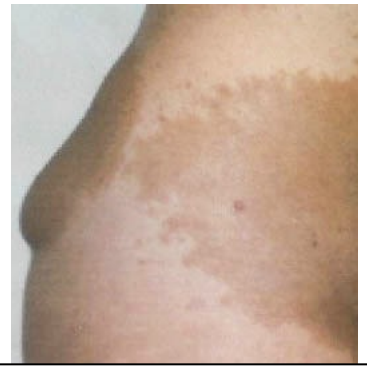
cutaneous



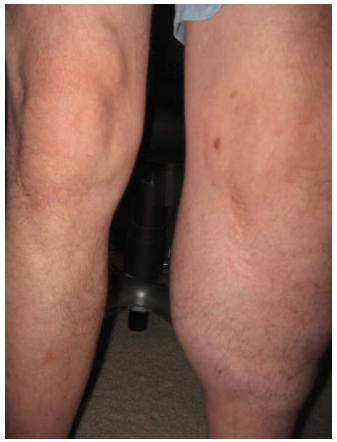


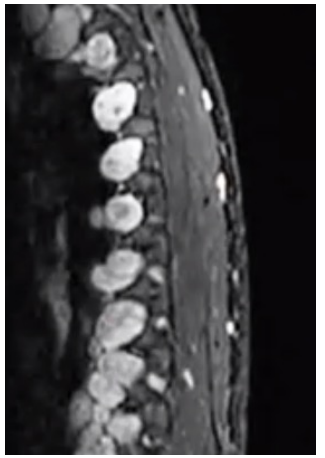


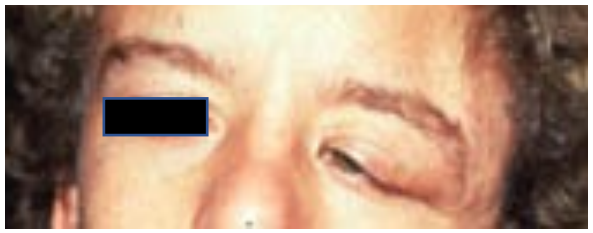
diffuse cutaneous



plexiform







Mouse studies suggest that the neurofibroma cell of origin is a Schwann cell precursor, which may be a boundary cap cell (which migrate throughout peripheral nervous system during development, and can differentiate into Schwann cells) (Li et al., *Neuro-Oncol Adv* 2020)

# NF1 – other characteristics

- ~1/3000 worldwide
- Homozygosity (constitutional *NF1* null) -> early embryonic lethal (assume patients heterozygous)
- People with NF1 at increased risk for other tumors/cancers, e.g. rhabdomyosarcoma, pheochromocytoma, breast cancer, glioblastoma (GBM), gastrointestinal stromal tumor (GIST).
- *NF1* among the most frequently mutated/ epigenetically silenced in sporadic cancers in people without NF1 (e.g. lung, GBM, melanoma, thyroid cancer, myeloid leukemia).
- >3500 known pathological variants (all types, scattered across gene). Microdeletion of *NF1* and flanking genes seen in ~4% of cases, all other pathological variant frequencies $\leq$ 1-2%.
- Only a few phenotype-genotype correlations thus far: variable expressivity.
- Symptoms progress variably with age, tend to be more similar in families, but unpredictable.
- Fully penetrant.
- Lifespan is 10 years less than population on average (likely from inclusion of MPNST deaths).
- Other features more common in NF1 than general population: learning disabilities, scoliosis, short stature, large head circumference, vasculopathy, seizures.
- Some patients report worsening of neurofibromas during puberty, pregnancy, and after age 65.

# NF1 Gene and Protein

- *NF1* (17q11.2): 60 exons (3 alternatively spliced) spanning 280 kb.
- Ubiquitously expressed at low levels (highest in neural crest tissues).
- Encodes neurofibromin (2818, 2839 amino acids - most common isoforms). **Tumor suppressor**.
  - Primary function is negative regulator of RAS pathway, by acting as a RAS GTPase-activating protein (GAP), facilitating conversion of active (GTP-bound) RAS proteins to inactive (GDP-bound) state. Encoded by middle eighth of molecule (**GRD** – GAP-related domain).
  - cAMP negative regulator.
  - other functions/domains based on homology or protein binding data (Figure below adapted from Bergoug et al., *Cells*, 2020)
- Cytoplasmic, and can be transported into the nucleus.

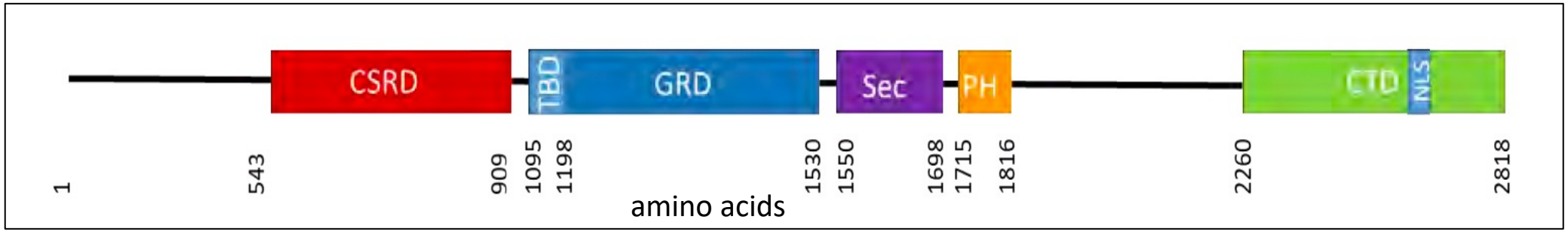
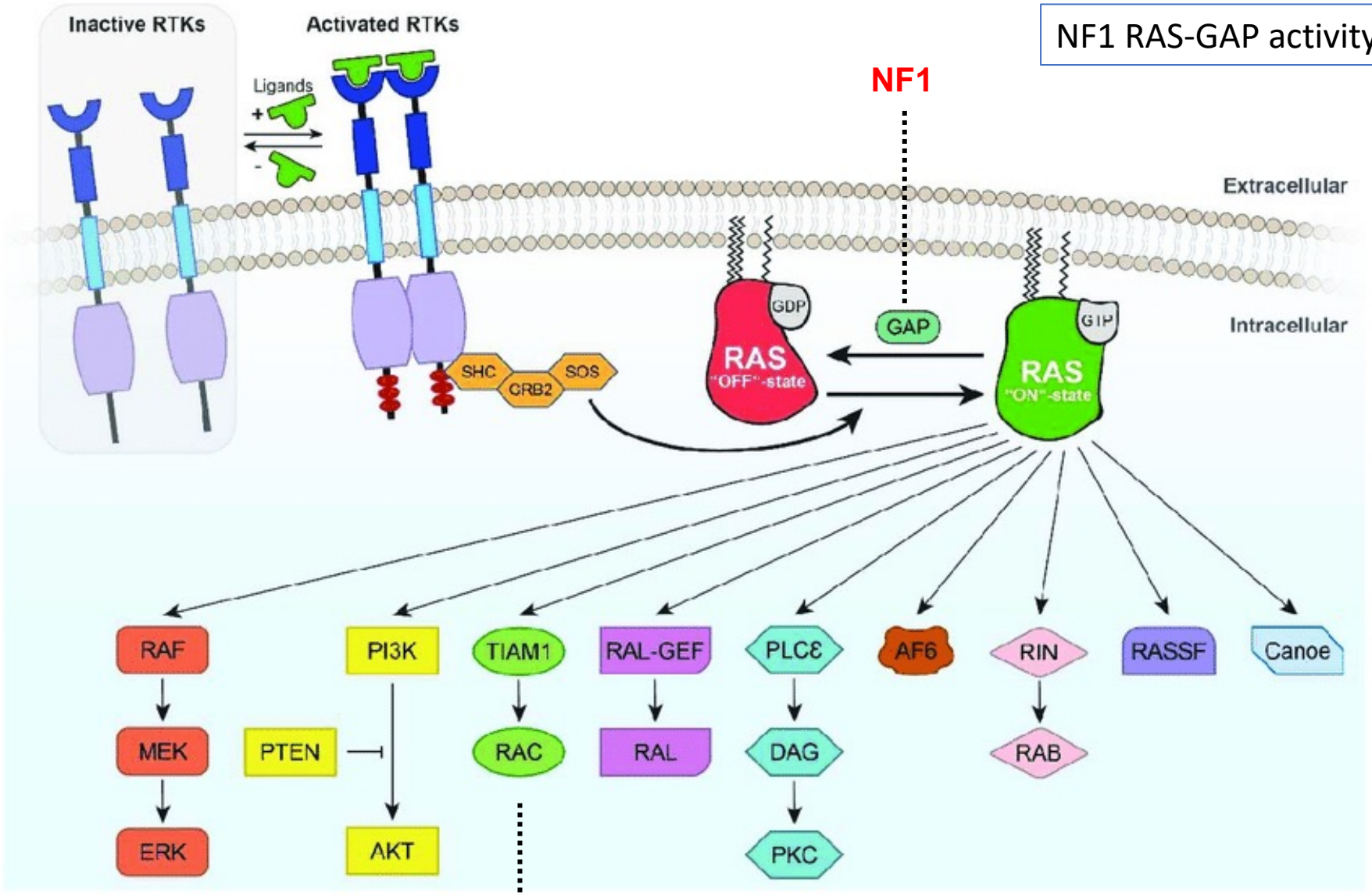



Figure 3. Schematic representation of neurofibromin domains. CSRD (cysteine- and serine-rich domain) in red, TBD (tubulin-binding domain) in light blue, GRD (GAP-related domain) in blue, Sec (Sec14 homologous domain) in purple, PH (pleckstrin homologous domain) in orange, CTD (C-terminal domain) in green, NLS (nuclear localization signal) in blue within CTD. Alt-spliced exon 23a encodes 21 amino acids starting at residue 1371 in GRD; its inclusion reduces GAP function.

NF1 RAS-GAP activity



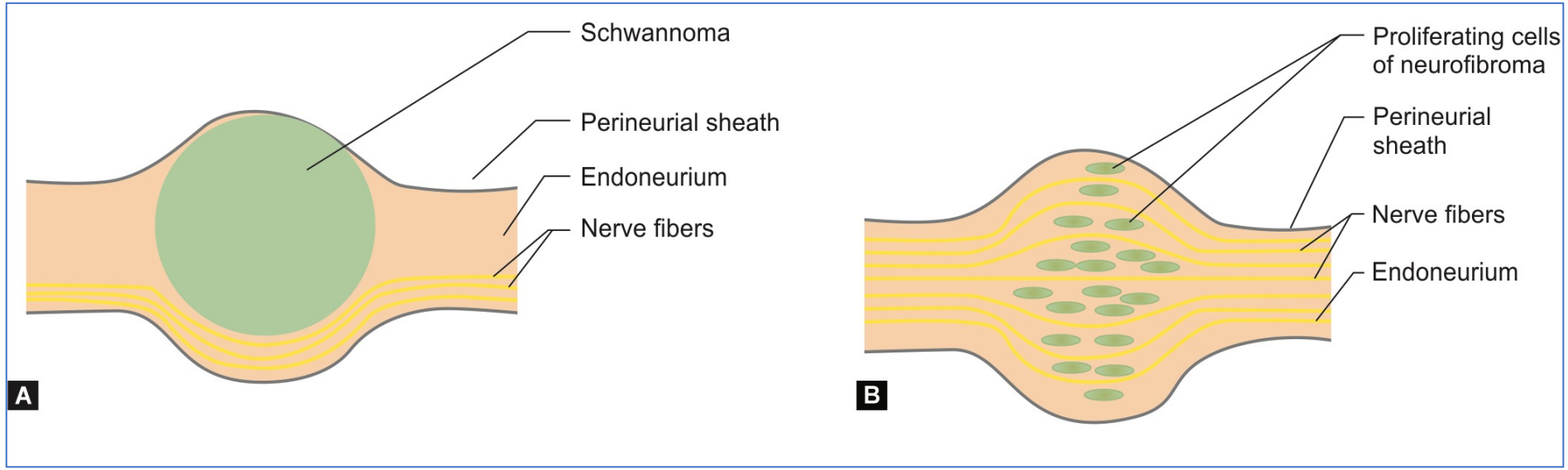

Adapted from Schöneborn et al., 2018, Intl J Mol Sci.

# NF1 mechanisms

- Neurofibromin – homodimer
- Mutant RNAs usually stable, at variably reduced levels (partial NMD).
- Unknown if truncated neurofibromin molecules are stable, but complete nullisomy for *NF1* (deletion of both alleles) is not seen in NF1 tumors, suggesting that some residual neurofibromin activity (at least amino terminus) is necessary for cell survival.
- Most mutations = reduced or loss of function, but some missense mutations (especially residues 844-848) appear to act in dominant negative fashion, with more severe clinical findings. Unknown whether in such cases, a somatic *NF1* mutation is still necessary.

# Schwannoma (left) versus neurofibroma (right)

(green = proliferating SC)

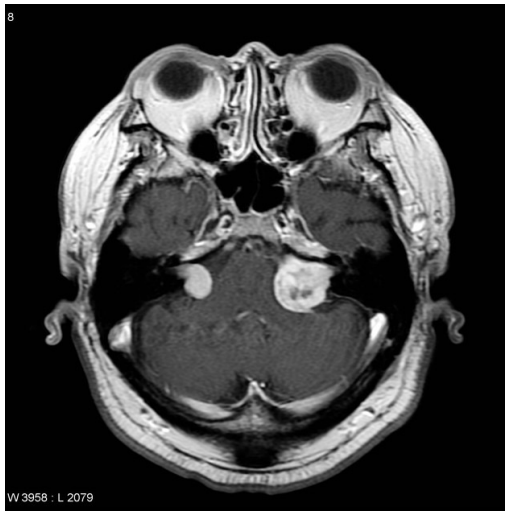



From Raghavendra Prasad et al., *Intl J Head Neck Surg*, 12(3), 2021.

Other cell types in these tumors: fibroblasts, perineurial cells, endothelial cells; hematopoietic cells are also present but in greater abundance in neurofibromas (mast cells, macrophages/CD34+ dendritic cells). Tumors contain variable amounts of extracellular matrix (typically collagen). [Histopathology: Stemmer-Rachamimov 2004 PMID: 15150133, Ortonne 2019 PMID: 32642737]

**Hybrid** tumors have histological features of both neurofibroma and schwannoma

# **NF2-SWN** Diagnostic Criteria




Gaillard F, NF2 Case study, Radiopaedia.org https://doi.org/10.53347/rID-5325

A diagnosis of NF2-related schwannomatosis can be made when an individual has **one** of the following:

1. Bilateral vestibular schwannomas (VS)(eighth cranial nerve)
2. An identical *NF2* gene pathogenic variant in at least 2 anatomically-distinct NF2-related tumors (schwannoma, meningioma, and/or ependymoma).
3. Either 2 major, or 1 major and 2 minor, criteria as described in the following:

   Major criteria:
   - Unilateral VS
   - First-degree relative other than sibling with NF2-related schwannomatosis
   - 2 or more meningiomas (note: a single meningioma qualifies as minor criteria).
   - *NF2* pathogenic variant in an unaffected tissue such as blood

   Minor criteria:
   - Can count >1 of a type (e.g., 2 distinct schwannomas would count as 2 minor criteria): ependymoma, meningioma (multiple meningiomas qualify as a major criteria), schwannoma. Note: if the major criterion is unilateral VS, at least 1 schwannoma must be dermal in location.
   - Can count only once (e.g., bilateral cortical cataracts count as a single minor criterion): juvenile subcapsular or cortical cataract, retinal hamartoma, epiretinal membrane in a person aged <40 years, meningioma.
   - Pattern of genetic changes in unaffected and tumor tissue in NF2-SWN: one *NF2* allele is a shared pathogenic variant in affected and unaffected tissues; the other *NF2* allele is wild type in unaffected tissues, and has different pathogenic variants (or loss of heterozygosity) in affected tissues.

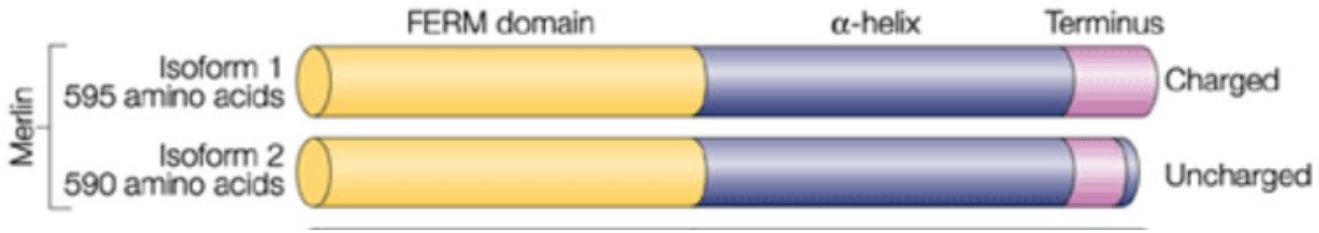
Plotkin et al., *Genet in Med*, 24:1967, 2022

# *NF2*-SWN other characteristics

- 1/28,000 births, one gene involved (*NF2)*
- Half of cases result from de novo *NF2* mutation in parent's gamete; 50% or more may actually have somatic mosaicism.
- Average age at diagnosis is 22 yrs: "adult" onset, but non-tumor characteristics may occur earlier
- Homozygosity (constitutional null) -> early embryonic lethal (assume patients heterozygous)
- >150 pathological variants reported (all types, scattered across gene).
- *NF2*-SWN patients at risk for progressive hearing and balance loss, decreased visual acuity, deficits usually due to intracranial and spinal tumors. Tumors confer high morbidity and lead to early mortality (despite being benign).
- Phenotype-genotype correlation is present: greater severity with earlier onset for truncating variants; milder/later onset for missense mutations and deletion of the whole locus; mixed effects from splicing mutations.
- Considered fully, or very nearly fully, penetrant.
- Most patients die prior to age 70, many by their 30s/40s.
- Malignant transformation of a tumor is possible, especially if it had received radiation therapy.

# *NF2*-SWN Gene and Protein, Mechanisms

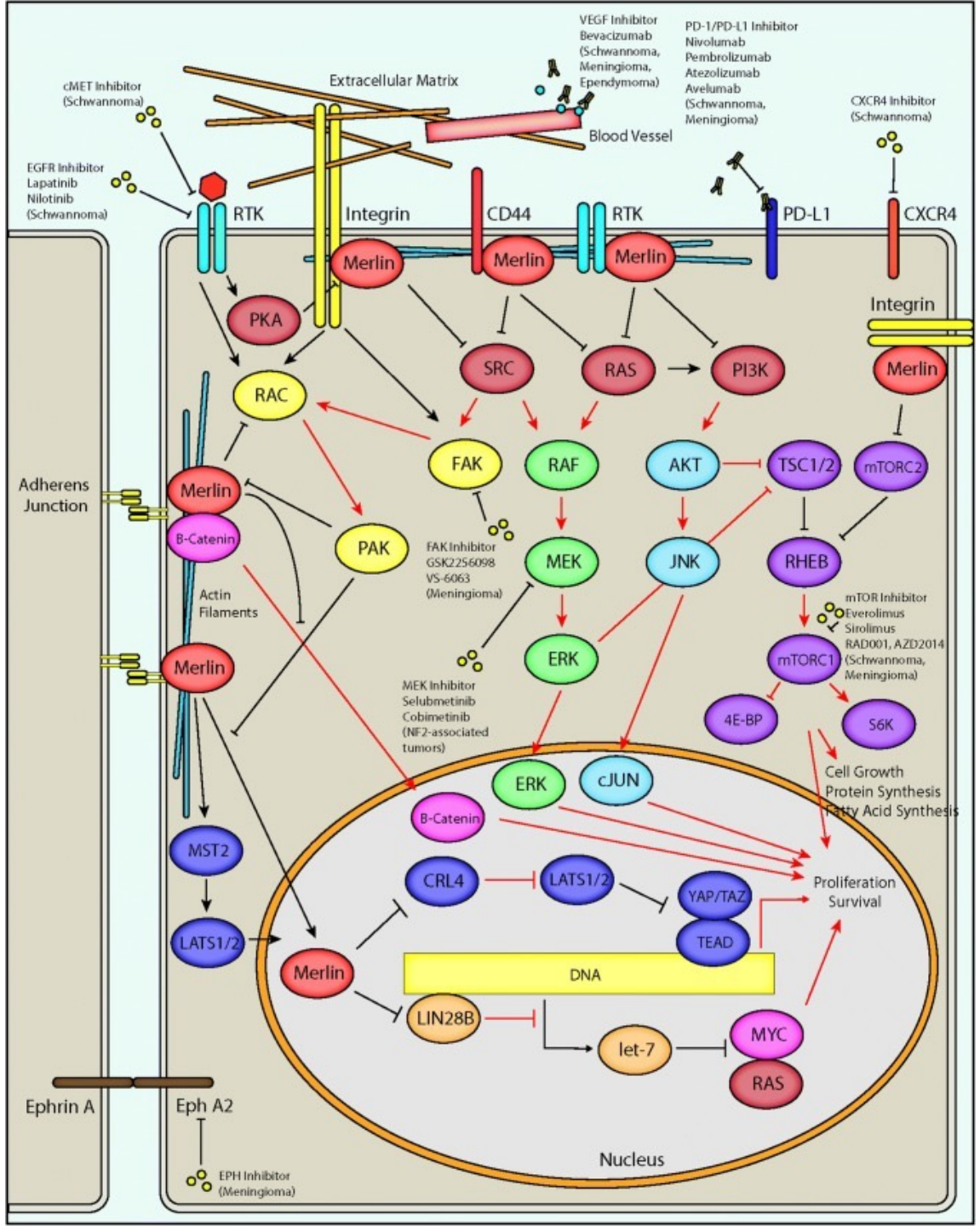
- *NF2* (22q12.1): 17 exons spanning 95 kb. Two major isoforms, based on alternative splicing of exon 16, affecting protein conformation. There are other, less common, isoforms as well.
- Ubiquitously expressed at low levels (highest in nervous systems).
- Encodes **merlin** (70 kD, 590 or 595 amino acids based on the major isoforms, Figure below), which mediates plasma membrane interactions with the cytoskeleton in polarized cells. This impacts multiple signal transduction pathways, particularly affecting the central and peripheral nervous systems. Member of band 4.1 superfamily (FERM domain). Merlin also plays a role in the trafficking of membrane proteins (both endo- and exo-cytosis).
- **Tumor suppressor:** two-hit mechanism in affected tissue (SC precursor in schwannoma, arachnoidal cells in meningioma, ependymal cells in ependymoma). No consistent additional somatic mutations.
- Loss of function variants, but there may be dominant negative mechanism too.
- Cytoplasmic and nuclear localization.




From Ramesh V. *Nat Rev Neurosci*, 2004.

Merlin

Pathways involving merlin as negative regulator: RAS (by blocking RAS-RAF binding), catenins/adherins (cell junctions), TGF-beta, mTORC1, SRC, RAC, NOTCH, CRL4-E3 ubiquitin ligase, and immune response pathways. Positive regulator of Hippo/MST/LATS/YAP and hedgehog paths.




Coy et al., Acta Neuropathol 139(4), 2020

# Schwannomatosis (**SWN**) Diagnostic Criteria (with *SMARCB1* or *LZTR1* variants)

A diagnosis of *SMARCB1*- or *LZTR1*-related SWN can be made when an individual meets 1 of the following criteria:

- At least 1 pathologically confirmed schwannoma or hybrid nerve sheath tumor and a *SMARCB1* (or *LZTR1*) pathogenic variant in an unaffected tissue such as blood
- A shared *SMARCB1* or *LZTR1* pathogenic variant in 2 schwannomas or hybrid nerve sheath tumors.
- Pattern of genetic changes in unaffected and tumor tissue in *SMARCB1*- and *LZTR1*-related SWN (see below; PV = pathogenic variant, LOH = loss of heterozygosity, *NF2* variants are somatic)

| Gene locus | Unaffected Tissue[c] | Tumor 1 | Tumor 2 | Comment |
|---|---|---|---|---|
| *SMARCB1/LZTR1* | | | | |
| Allele 1 | PV1[d] | PV1 | PV1 | Shared *SMARCB1* or *LZTR1* pathogenic variant |
| Allele 2 | WT | LOH | LOH | Tumor-specific partial loss of 22q in trans position, LOH typically entails deletion of 22q region encompassing *LZTR1/SMARCB1/NF2* |
| *NF2* | | | | |
| Allele 1 | WT | PV2 | PV3 | Tumor-specific pathogenic *NF2* variant in cis to pathogenic *SMARCB1* variant |
| Allele 2 | WT | LOH | LOH | Tumor-specific partial loss of 22q in trans position, LOH typically entails deletion of 22q region encompassing *LZTR1/SMARCB1/NF2* |

Variants that are not classified as pathogenic (Benign, Likely Benign, Uncertain) cannot be used for diagnosis

Plotkin et al., *Genet in Med*, 24:1967, 2022

# Other **SWN** Diagnostic Criteria (no germline mutations in *SMARCB1, LZTR1, DGCR8*)

**Table 3** Revised diagnostic criteria for schwannomatosis in persons with no pathogenic variants in blood but with loss of chromosome 22q in multiple schwannomas

Diagnostic criteria for 22q-related schwannomatosis

A diagnosis of 22q-related schwannomatosis can be made when an individual does not meet criteria for *NF2*-related schwannomatosis, *SMARCB1*-related schwannomatosis, or *LTZR1*-related schwannomatosis, does not have a germline *DGCR8* pathogenic variant, and has both of the following molecular features:

- LOH of the same chromosome 22q markers in 2 anatomically distinct schwannomas or hybrid nerve sheath tumors and
- A different *NF2* pathogenic variant in each tumor, which cannot be detected in unaffected tissue

Pattern of genetic changes in unaffected and tumor tissue in 22q-related schwannomatosis[a]

| | Unaffected Tissue[b] | Tumor 1 | Tumor 2 | Comment |
|---|---|---|---|---|
| *SMARCB1/ LZTR1* | | | | |
| Allele 1 | WT | None found | None found | No shared pathogenic *LZTR1* or *SMARCB1* variant |
| Allele 2 | WT | LOH | LOH | Tumor-specific partial loss of the same chromosome 22q, LOH typically entails deletion of 22q region encompassing *LZTR1/SMARCB1/NF2* |
| *NF2* | | | | |
| Allele 1 | WT | PV1 | PV2 | Tumor-specific pathogenic *NF2* variant trans to the 22q deletion |
| Allele 2 | WT | LOH | LOH | Tumor-specific partial loss of the same chromosome 22q, LOH typically entails deletion of 22q region encompassing *LZTR1/SMARCB1/NF2* |

*LOH*, loss of heterozygosity; *PV1*, pathogenic variant; *WT*, wildtype.

[a]See also Supplemental Figure 1C.

[b]Tissues unaffected by tumors, such as blood or skin.

Plotkin et al., *Genet in Med*, 24:1967, 2022

# **SWN** Other Characteristics

- 1/69,000 births (may be an under-estimate), associated with germline mutations in ≥2 genes (tumor suppressors), predominantly *SMARCB1* (part of SWI/SNF chromatin remodeling complex) and *LZTR1* (encodes a protein in Golgi apparatus)
- ~1/3 patients have tumors limited to one part of body (somatic mosaic, "segmental")
- No cutaneous manifestations.
- Somatic (e.g. tumor) genetics is complex because multiple genes/alleles involved.
- Localized and/or diffuse pain is primary symptom (not always related to a tumor), but tumors can also cause neurologic deficits. Chronic pain can lead to anxiety and/or depression.
- Age at diagnosis is usually teens - 30s
- At risk for meningioma as well (5%), especially if there is a *SMARCB1* mutation
- Phenotype-genotype correlations are still being characterized. Some *SMARCB1* germline mutations are associated with rhabdoid-type tumors (Rhabdoid Tumor Predisposition Syndrome) instead of schwannomas (not SWN), or with another allelic disorder, Coffin-Siris syndrome. Mutations in *LZTR1* can also cause Noonan syndrome type 10. There is clinical overlap with other conditions as well.
- Considered to have incomplete penetrance (Plotkin et al., *Genet in Med*, 24:1967, 2022).
- Malignant transformation of a schwannoma (to MPNST) is rare.
- Average lifespan is slightly below that of the general population.
- Mechanism is complex: loss-of-function at multiple loci (2-hit). Gain-of-function mechanism in some cases/alleles has been proposed but not proven functionally.
- Non-penetrance and mosaicism can complicate diagnosis and genetic counseling.

# Preclinical Resources

Cells: human wild type (+/+), *NF1* +/1 and -/- SC (neurofibroma) cultures and lines (some immortalized) are available at ATCC and ABM (abmgood.com) (some listed at https://www.n-tap.org/for-researchers/cell-model-systems). Some labs have made cell lines from animal models as well. Fewer such cell resources for NF2-SWN and SWN currently, although some have been created through genome editing of +/+ SC. iPSCs from patients have been created by several labs, can be differentiated and used in organoid culture. Check Cellosaurus and literature, and reach out to NF investigators.

Animals: (check Jackson Laboratories, and reach out to NF investigators)
NF1: A number of mouse lines recapitulating one or more *NF1* human mutations and/or clinical features have been created, through genome editing and/or conditional knockout technologies. *NF1* +/- constitutional mice do not show NF1 features. (see partial list at https://www.n-tap.org/for-researchers/animal-models). There are also now rat, pig and zebrafish models. Drosophila is also a model organism used. Also, orthotopic xenografts with human *NF1* -/- cells is used.

NF2-SWN: somewhat fewer models. *NF2* +/- constitutional mice do not show NF2-SWN features, thus specific engineering has been required as for NF1. Mouse models recapitulate schwannoma (including VS) and meningioma. Zebrafish and Drosophila models have also been developed. There is also an NF2-SWN monkey model. Orthotopic xenograft with human tumor cells is also used.

SWN: requires manipulation of one gene for germline background, and creation of somatic mutation at 2 loci (Vitte et al., *Nat Commun* 8(1), 2017). Orthotopic xenografts with human tumor cells are also used.

# Considerations for Gene Therapy for Neurofibromatoses

Cell/tissue target (constitutional, or specific tissue, tumor or other manifestation)
DNA or RNA target (germline vs somatic variants, and/or epigenetic alterations)
Mechanisms involved (dictates approach – replacement, correction, etc)
    If replacement, consider impact of level of transgene expression
Delivery vehicle for target cell/tissue (e.g. groups screening for AAVs with SC tropism)
Outcome measures (given that these conditions usually progress slowly)
Level of efficacy considered to be a therapeutic effect
Durability of treatment (lifelong depending on what is targeted) and option to re-treat
Genetic and clinical heterogeneity (two-hit affected tissues are independent)
Preclinical model options
Prevention vs treatment, and most effective age(s) for intervention
Risk vs benefit (disorders are not life-threatening in many patients)
Potential production feasibility

# Suggested References/ sources

https://www.ctf.org/understanding-nf/ and https://www.ctf.org/research-tools-resources

https://www.uptodate.com/contents/neurofibromatosis-type-1-nf1-pathogenesis-clinical-features-and-diagnosis

https://www.uptodate.com/contents/nf2-related-schwannomatosis-formerly-neurofibromatosis-type-2?topicRef=2939&source=see_link

https://www.uptodate.com/contents/schwannomatoses-related-to-genetic-variants-other-than-nf2?topicRef=5206&source=see_link

Single cell sequencing (and see DATA source below): PMID: 34053190, PMID: 36134665, PMID: 33413690

https://www.ncbi.nlm.nih.gov/books/NBK1201/

https://www.ncbi.nlm.nih.gov/books/NBK1109/

https://www.ncbi.nlm.nih.gov/books/NBK487394/ and Evans et al., 2018, PMID: 29909380 and Mansouri et al., 2021, PMID: 33025139

DATA source: NF Data Portal (Synapse, housed by Sage Bionetworks): nfsynapse.org, and https://www.synapse.org (search “neurofibromatosis” or related term).