Updates from the International NF-SWN Genes Variant Curation Expert Panel (VCEP) to Improve Genetic Testing of Neurofibromatosis and Schwannomatosis

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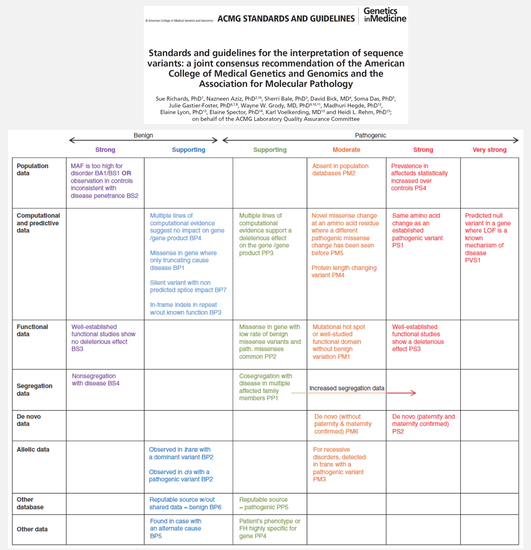
BACKGROUND

Neurofibromatosis type 1 (NF1); *NF2*-, *SMARCB1*-, and *LZTR1*-related schwannomatosis (SWN); and Legius syndrome (LGSS) require a genetic diagnosis (1) to confirm clinical suspicion in patients with indeterminate phenotype, (2) to better understand a patient's prognosis and (3) for family planning. Variant interpretation and classification of the five genes causing these disorders (*NF1*, *NF2*, *SMARCB1*, *LZTR1*, and *SPRED1*) is challenging due to the broad mutational spectrum, the paucity of clear mutational "hotspots", and the high proportion of non-coding and splicing variants. Many of the patients do not have a family history due to high *de novo* and mosaicism rate and/or variable penetrance and expressivity and do not meet diagnostic criteria in the early stages of these disorders. These patients would benefit from an accurate genetic test for clinical use and follow-up.

METHODS

Thirty-seven individuals from North and South America, Australia, and Europe with expertise in NF1, LGSS, and SWN (or other related hereditary tumor predisposition pathologies) or from high-volume diagnostic laboratories (academic and commercial) volunteered to develop ACMG/AMP variant interpretation rules specific for NF-SWN genes as members of a Variant Curation Expert Panel (VCEP) in the framework of the NIH-funded ClinGen Hereditary Cancer Clinical Domain Working Group. This panel of experts includes clinical and molecular geneticists, variant scientists, genetic counselors, epidemiologists, neurosurgeons, and others who regularly participate in the diagnosis and/or clinical management of this group of disorders.


ACMG STANDARDS AND GUIDELINES | Genetics in Medicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

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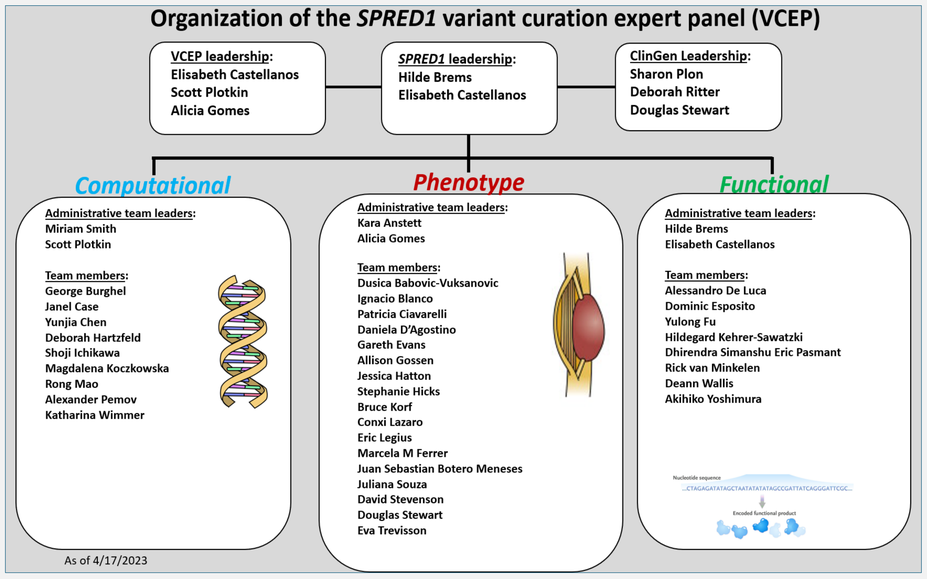
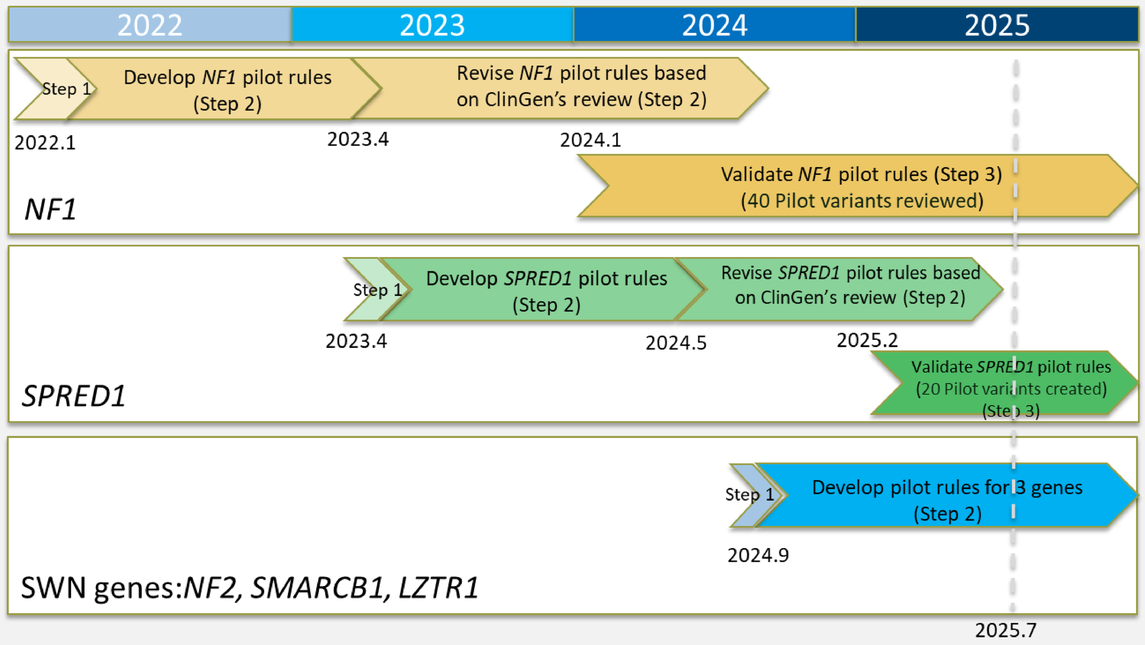
	Benign	Pathogenic				
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data → benign BP6	Reputable source → pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

RESULTS

The NF-SWN Genes VCEP is comprised of five sub-VCEPs that will address causative genes associated with NF1 (*NF1*), LGSS (*SPRED1*), and SWN (*NF2*, *SMARCB1*, and *LZTR1*). For each disorder, a sub-VCEP has been organized into three working groups (functional, phenotypic, and computational) to review and modify, if required, the 26 general ACMG/AMP rules to establish specific criteria for each gene.





The NF-SWN Genes VCEP has successfully developed the first version of *NF1* and *SPRED1* ACMG/AMP guidelines and is close to completing the pilot study of *NF1* rules. A pilot study of *SPRED1* rules has begun recently. Furthermore, the establishment of SWN rules is underway, in conjunction with the analysis of *NF2*, *SMARCB1*, and *LZTR1* gene codes.

MODIFICATION EXAMPLES

1. Apply the scaled point system to the ACMG/AMP variant classification guidelines

Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

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Fitting a naturally scaled point system to the ACMG/AMP variant classification guidelines

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TABLE 2 Point values for ACMG/AMP strength of evidence categories

Evidence Strength	Point scale Pathogenic	Benign
Indeterminate	0	0
Supporting	1	−1
Moderate	2	−2
Strong	4	−4
Very strong	8	−8

TABLE 3 Point-based variant classification categories

Category	Point ranges
Pathogenic	≥10
Likely Pathogenic	6 to 9
Uncertain	0 to 5
Likely Benign	−1 to −6
Benign	≤ −7

2. Integrate scaled point system to certain criteria, such as PS4 (prevalence in affected individuals over controls)

PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls

Note 1: Relative risk or OR, as obtained from case–control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0. See the article for detailed guidance.

Note 2: In instances of very rare variants where case–control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

For the *NF1* gene, evidence strength is categorized into 4 levels, ranging from supporting (1-1.5 points), moderate (2-3.5 points), strong (4-15.5 points), to very strong (>=16 points), based on sum points accumulated from unrelated probands.

- Apply 0.5 points to each proband with a moderately-specific phenotype, and 1 point to each proband with a highly-specific phenotype.
- If an individual is noted to meet NIH criteria in publication without phenotypic features provided, full points should be applied.
- If an individual is noted to meet NIH criteria in internal data without phenotypic features provided, half points should be applied.
- Do not apply PS4 at any strength if BA1 orBS1 is met.

TAKE HOME POINTS

- The NF-SWN Genes VCEP has been established with the objective of refining specific ACMG/AMP rules for use in curating NF-SWN gene variants
- The primary aim of this initiative is to develop a compendium of NF-SWN gene-specific ACMG/AMP evidence rules
- The eventual goal of this endeavor is to ensure more accurate variant interpretations for clinical use in the context of NF and SWN patients.

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