DETECTION OF *NF1* VARIANTS BY NGS PANELS WITH SNV AND CNV IDENTIFICATION EXCEEDS PUBLISHED ESTIMATES AND IMPROVES CLINICAL UTILITY

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BACKGROUND

- Neurofibromatosis type 1 (NF-1) is an autosomal dominant condition due to loss-of-function mutations in the neurofibromin 1 (*NF1*) gene. NF-1 affects individuals of all ethnicities and has a prevalence of 1 in 3,500.
- Nearly half of all NF-1 cases are caused by de novo sporadic mutations in the *NF1* gene. The variant landscape of pathogenic *NF1* variants includes missense and nonsense substitutions, splice variants, small insertions and deletions, sub-genic copy number variants (CNVs), as well as whole-gene deletions.
- Next-generation sequencing (NGS) panels have enabled dramatic improvements in the detection of nearly all of these variants simultaneously. Current published literature estimates that sequencing genomic DNA (gDNA) can detect *NF1* variants in 60-90% of individuals, while combined cDNA and gDNA sequencing enables detection of >95% of *NF1* variants.
- Current recommendations for *NF1* variant detection appear to undervalue the clinical utility of gDNA analysis in NGS panels and overstate the relative importance of cDNA analysis.

METHODS

- We conducted a literature review of published studies assessing the *NF1* variant landscape and found that 97.5% of the variants reported in NF-1 patients would be detectable by NGS sequencing using gDNA exclusively and analyzing exonic and canonical splice site intronic regions. The only variants not detectable by gDNA analysis but detected by cDNA analysis were deep intronic variants, which comprised just 2.5% of all *NF1* variants reported to date and were shown or predicted to create novel splice sites (Table 1).
- We also performed an analysis of *NF1* variants identified among 490 patients referred for NF-1 testing at Invitae. We detected an *NF1* variant in all patients described by their ordering physicians as exhibiting clinical features of NF-1. Patient features included a clinical or suspected NF-1 diagnosis, or café-au-lait spots, inguinal/axillary freckling, plexiform neurofibromas, optic glioma, Lisch nodules, and/or osseous lesions (pseudarthrosis, scoliosis).
- We used our Sherloc algorithm[1] for semi-quantitative variant classification, based on ACMG guidelines, to interpret detected variants.
- Given that 50% of NF-1 cases are sporadic, we performed targeted family variant testing (FVT) to resolve the clinical significance of any variants of uncertain significance (VUS). Of 16 families that enrolled in FVT, 13 were for suspected de novo, and 3 were for suspected segregation. In 2 families, only 1 parent was available for testing and de novo status was not confirmed.

RESULTS

- An *NF1* variant was detected in all patients with NF-1 features.
  - 69.38% of *NF1* variants were pathogenic (P), 13.28% were likely pathogenic (LP), and 17.34% were variants of uncertain significance or VUS (Figure 1).
  - Among the detected *NF1* variants, 94% were in coding regions or the consensus splice site, with the remaining 6% in adjacent intronic regions (Figure 2).
  - Most VUS in *NF1* were missense variants (>75%), while intronic variants near intron-exon junctions comprised 13%. In-frame indels, silent variants, and sub-genic duplications comprised under 10% of VUS combined (Figure 3).

Figure 1. *NF1* variant classification in 490 patients with NF-1 clinical features.

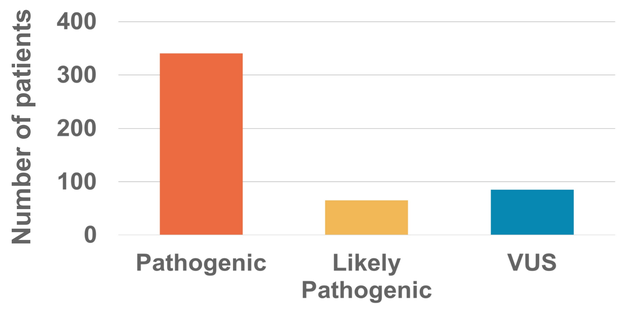


Figure 2. Effect of all gDNA *NF1* variants detected.

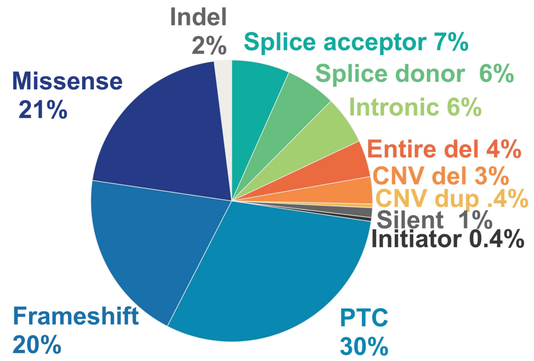
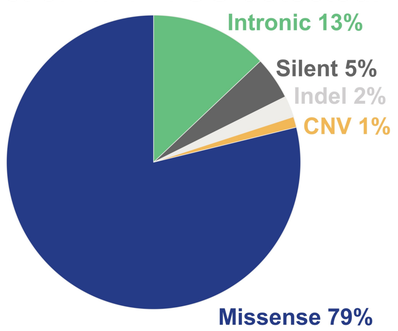


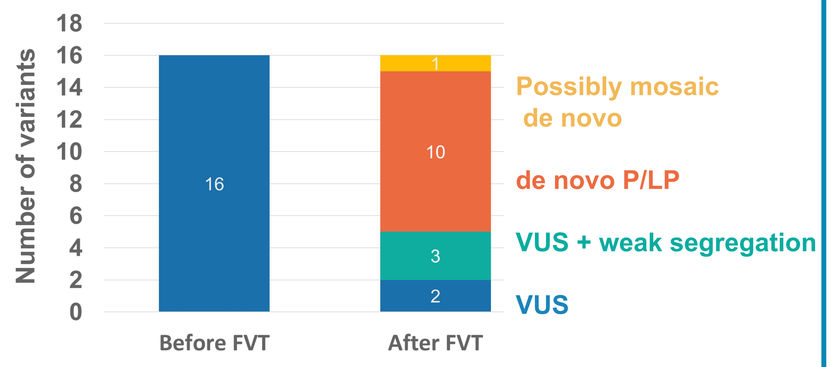
Figure 3. Effect of *NF1* VUS detected.



RESULTS

- Among families that elected to undergo FVT for VUS resolution, 11/13 suspected de novo cases were confirmed to be de novo, one of which was possibly mosaic (Figure 4). In 2/13 families with suspected de novo variants, only 1 parent was available for FVT and de novo status could not be confirmed.
- The remaining 3 family variants remained VUS, but with increased evidence toward pathogenicity based on segregation of the variant in the families.

Figure 4. Variant classification before and after family variant testing (FVT).



CONCLUSIONS

- **NGS-based *NF1* gDNA sequencing with both SNV and CNV detection identifies > 95% of variants reported in the literature.**
- **Intronic and deep intronic variants outside the typical reportable range of exonic and canonical splice site regions account for 2.5% of *NF1* variants reported in the literature.**
- **While RNA/cDNA analyses provide additional functional data, this information alone is generally unlikely to alter variant classification. NGS gDNA panels offer nearly comparable detection to cDNA approaches.**
- **Missense variants comprise a greater proportion of *NF1* VUS than intronic variants, and allow for the classification of more actionable variants by FVT at scale.**
- **Among individuals with NF-1 and negative family history, FVT confirmed de novo status and resolved VUS in 11/13 or 85% of patients.**
- **Family studies for segregation and de novo status provide powerful evidence that Invitae NGS panels are a strong clinical option for *NF1* variant testing.**

Table 1. Literature review of published variants in NF-1 cases with both gDNA and cDNA sequenced.

PMID	Cohort size	Total variants detected	Variants in CDS	Intronic +/- 10 bp of exon	Deep intronic	Sub-genic CNV	Whole-gene CNV
23913538	565	546	432	101	13	22	24
21354044	105	93	79	2	2	5	10
27322474	361	348	262	13	9	7	21
Total	1,031	987	773	116	24	34	55
Percentage			78.32%	11.75%	**2.43%**	3.44%	5.57%

Reference: 1. Nykamp K, Anderson M, Powers M, Garcia J, Herrera B, Ho Y-Y *et al.* Sherloc: A comprehensive refinement of the ACMG-AMP variant classification criteria. *Genet Med.* 2017;19(10):1105–17. http://doi.org/10.1038/gim.2017.37

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