Low Variant Allele Fraction in Germline Genetic Testing Predicts Pathogenicity of *NF1* Variants

Shoji Ichikawa, PhD, Timothy Komala, BS, Meghan Towne, MS, CGC
Ambry Genetics, Aliso Viejo, California
sichikawa@ambrygen.com

SCAN ME

BACKGROUND

Patients with a clinical diagnosis or suspicion of neurofibromatosis type 1 (NF1) undergo germline genetic testing for the *NF1* gene. *NF1* is also included in multigene panel testing for cancer predisposition and neurodevelopmental disorders.

Low variant allele fraction (VAF) in *NF1* variants is frequently observed in germline genetic testing (next-generation sequencing, NGS) using blood or saliva DNA and may result from mosaic/segmental NF1, circulating tumor DNA, or clonal hematopoiesis of indeterminate potential.

Clonal hematopoiesis can be caused by somatic mutations and increases with age.[1] *NF1* may provide a selective advantage to hematopoietic stem cells when mutated, thereby driving clonal hematopoiesis.

METHODS

Rare *NF1* variants (total allele frequency <0.1% in gnomAD v2) were identified in patients who submitted blood or saliva for germline genetic testing from 2016 to 2023.

To ensure sufficient coverage and minimize technical interference in variant calling, only single nucleotide substitutions in coding sequence and within +/- five nucleotides from exons were included in analyses. VAF <10% was filtered out to exclude potential sequencing artifacts.

VAF in blood or saliva was available for 4,145 unique *NF1* variants detected in 43,457 patients (7,688 males, 35,718 females, and 51 unknowns):

- 387 likely pathogenic/pathogenic (LP/P)
- 2,263 variant of unknown significance (VUS)
- 1,495 likely benign/benign (LB/B)

Classification was based on the current ACMG/AMP standards and guidelines for the interpretation of sequence variants[2] in conjunction with an internally developed protocol available at the time of variant assessment (https://www.ambrygen.com/science/variant-classification).

There was no statistical difference in *NF1* VAF between blood and saliva (data not shown), and thus, both specimen types were combined for the subsequent analyses.

Low VAF was defined as three standard deviations below the mean in patients with LB/B variants (35.1%).

Table 1. Frequencies of unique *NF1* variants observed at low VAF in germline genetic testing. Low VAF occurred with all classifications, but it is most frequently detected with LP/P variants.

Classification	Number of unique variants	Number of unique variants observed at low VAF	Frequency of low VAF (%)
LP/P	387	128	33.1
VUS	2,263	189	8.4
LB/B	1,495	125	8.4
Total	**4,145**	**442**	**10.7**

RESULTS

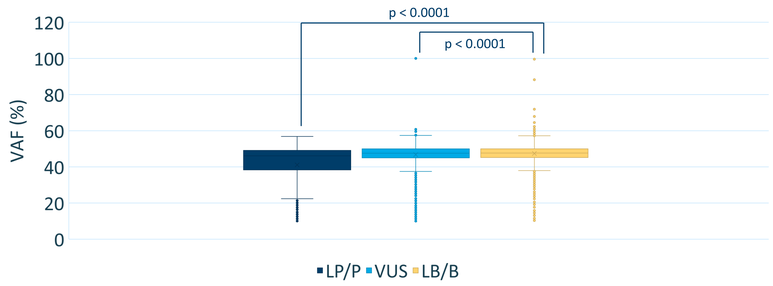

Figure 1. Mean VAF of rare *NF1* variants observed in blood and saliva (880 patients with LP/P, 6,544 patients with VUS, and 36,033 with LB/B). Mean VAF were significantly different among patients with LP/P, VUS, and LB/B – 41.1%, 46.8%, and 47.4%, respectively (T-test p-value < 0.0001, compared to LB/B).

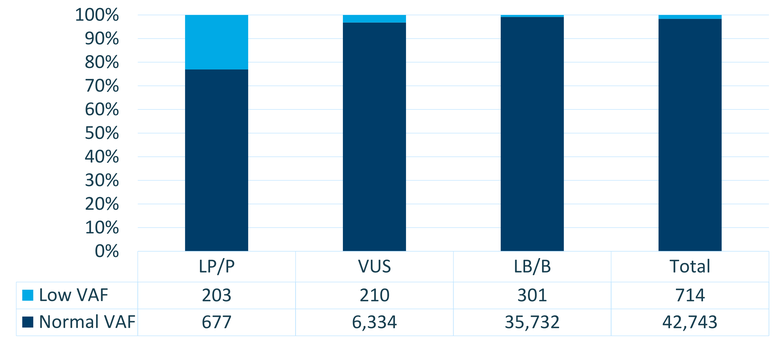

Figure 2. Detection of low-VAF *NF1* variants in patients undergoing germline genetic testing. 1.6% (714/42,743) of the patients had low VAF in *NF1*. A significantly higher proportion of the LP/P variants was detected at low VAF – 23.1% with LP/P and 0.8% with LB/B (Fisher's exact test p-value < 0.0001).

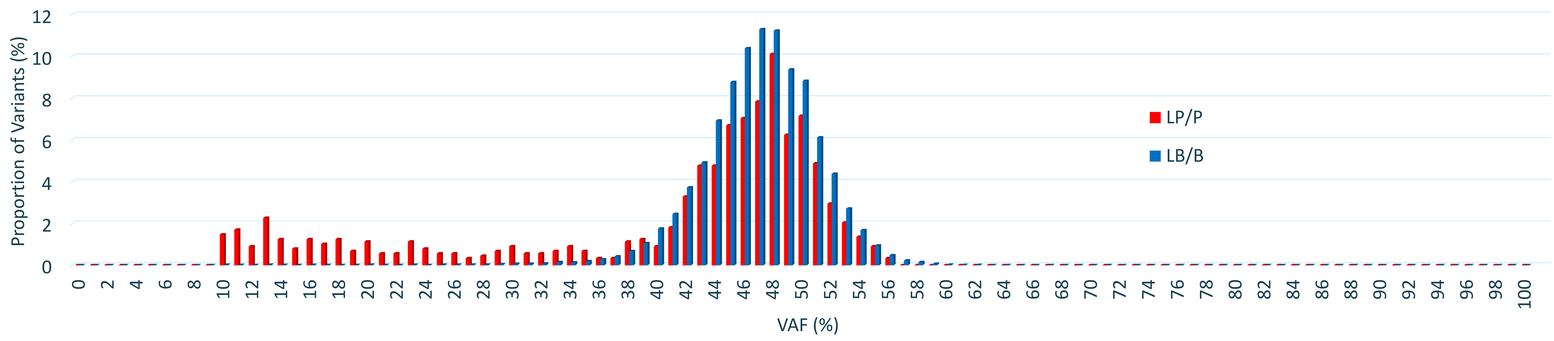

Figure 3. VAF distributions of rare *NF1* variants in blood and saliva (880 patients with LP/P and 36,033 with LB/B). VAF of both LP/P and LB/B variants peaked near 50% as expected for heterozygotes. However, a substantial number of patients with LP/P variants had VAF lower than expected.

Table 2. Mean ages of patients with rare *NF1* variants. Patients with LP/P variants were significantly younger than those with LB/B variants likely because the LP/P group has a large proportion of pediatric patients with a clinical diagnosis or suspicion of NF1. In contrast, mean age of patients with low VAF was significantly higher for LP/P and VUS, compared to LB/B.

Classification	Mean age of patients with rare *NF1* variants, years (N)	Mean age of patients with low-VAF *NF1* variants, years (N)
LP/P	44.1* (877)	61.1* (201)
VUS	53.1 (6,534)	61.7 *(209)
LB/B	53.0 (35,913)	54.0 (301)
Total	**52.8 (43,327)**	**58.3 (714)**

**T-test p-value < 0.0001, compared to mean age of patients with LB/B variants

TAKE-HOME POINTS

- 1.6% of rare *NF1* variants detected in blood and saliva DNA had low VAF in germline genetic test (NGS)
- Although low VAF was observed with LB/B variants in *NF1*, it was significantly associated with LP/P variants
- Low VAF was more prevalent in older patients with LP/P variants, suggesting that *NF1* pathogenic variants may drive age-related clonal hematopoiesis
- Our data indicates that low VAF can predict pathogenicity of *NF1* variants and provide *in vivo* functional evidence to aid classification of *NF1* variants
- As much as 8% of *NF1* VUS observed with low VAF may benefit from reassessment

REFERENCES

1. Weeks and Ebert. *Blood*. 2023 Dec 28;142(26):2235-2246.
2. Richards et al. *Genet Med*. 2015 May;17(5):405-24.