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

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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) using blood or saliva and may result from mosaic/segmental NF1, circulating tumor DNA, or clonal hematopoiesis of indeterminate potential. *NF1* may provide a selective advantage to hematopoietic stem cells when mutated, thereby driving clonal hematopoiesis. In this study, we sought to determine whether low VAF is associated with pathogenicity of *NF1* variants.

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 *NF1* variants in 43,457 patients – 387 likely pathogenic/pathogenic (LP/P), 2,263 variant of unknown significance (VUS), and 1,495 likely benign/benign (LB/B). *NF1* VAF was compared between blood and saliva, as well as between LP/P and LB/B variants. Low VAF was defined as three standard deviations below the mean in patients with LB/B variants (35.1%).

Results: There was no statistical difference in *NF1* VAF between blood and saliva, and thus, both specimen types were combined for the subsequent analyses. Mean VAF of rare *NF1* variants was significantly different among patients with LP/P, VUS, and LB/B – 41.1%, 46.8%, and 47.4%, respectively. VAF of both LP/P and LB/B variants peaked near 50% as expected for heterozygotes. However, a significantly higher proportion of the patients with LP/P variants had low VAF – 23.1% with LP/P and 0.84% with LB/B. Low VAF was observed with 8.4% (189/2,263) of the VUSs.

Conclusions: Rare *NF1* LP/P variants were significantly associated with low VAF in blood and saliva. While the exact cause of low VAF may be unknown, *NF1* pathogenic variants may be driving clonal hematopoiesis in patients undergoing germline genetic testing. Our data indicates that low VAF can predict pathogenicity of *NF1* variants and provide *in vivo* functional evidence to aid classification of *NF1* variants. *NF1* VUS observed with low VAF may benefit from reassessment.

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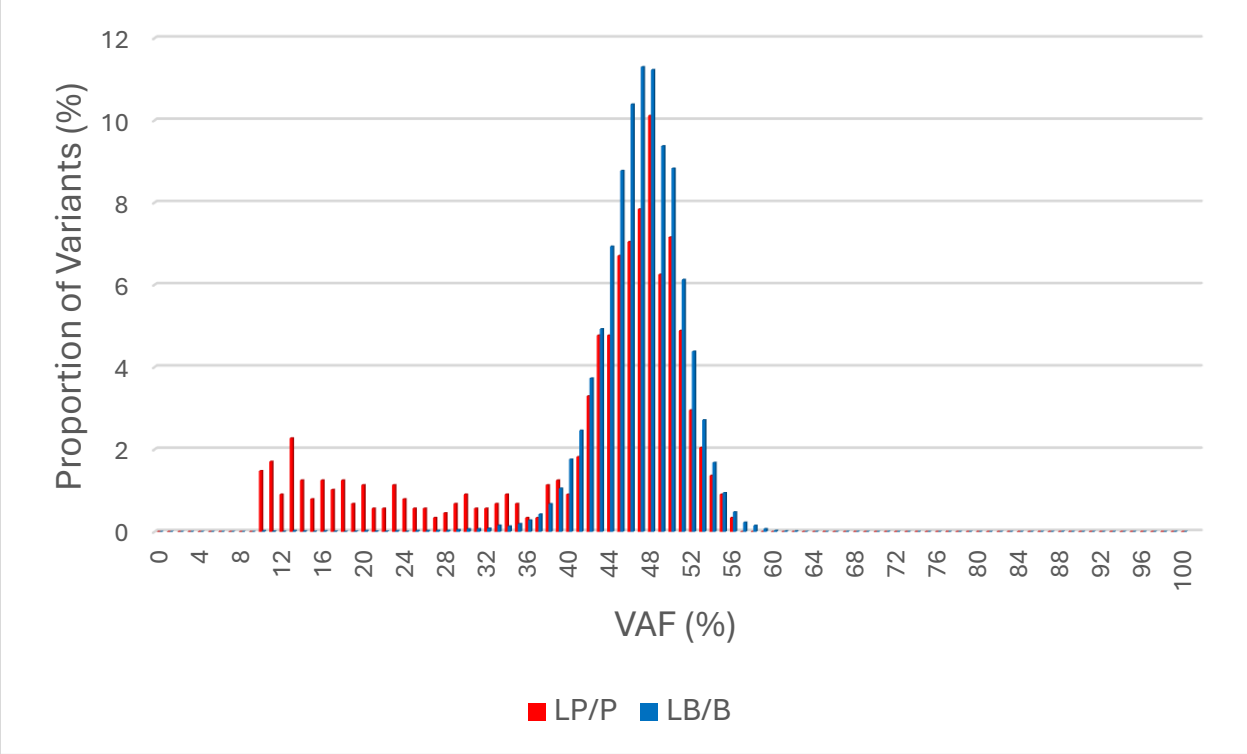

Figure 1. VAF distributions of rare *NF1* variants in blood and saliva (880 patients with LP/P and 36,033 with LB/B). A significantly higher proportion of the patients with LP/P variants had low VAF, compared to those with LB/B variants (Fisher's exact test p-value < 0.0001).