Fractional Clues: Decoding *NF1* Variant Pathogenicity Through Low VAF

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Introduction:

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder. Low variant allele fraction (VAF) in *NF1* variants is frequently observed in germline genetic testing using next-generation sequencing. Low VAF can result from biological phenomena such as post-zygotic mosaicism and clonal hematopoiesis, or from technical artifacts. In age-related clonal hematopoiesis, somatic pathogenic variants acquired during aging confer a selective advantage to hematopoietic stem cells, leading to the expansion of mutant clones. The clinical significance of low VAF in *NF1* variants remains unclear, especially regarding its association with pathogenicity. This study investigates whether low VAF predicts pathogenicity of *NF1* variants and applies the Bayesian ACMG framework to quantify the evidence strength of low VAF for variant classification.

Methods:

Rare *NF1* variants (total allele frequency <0.1% in gnomAD v2) were identified in patients who underwent germline genetic testing (blood or saliva) from 2016 to 2023. To ensure sufficient coverage and minimize technical artifacts, analysis included only single-nucleotide substitutions in coding regions and adjacent intronic sequences and excluded variants detected with VAF <10%. *NF1* variants meeting the selection criteria were identified in 43,457 patients. Variants were classified as likely pathogenic/pathogenic (LP/P), variant of uncertain significance (VUS), or likely benign/benign (LB/B) according to the ACMG/AMP guidelines and internal protocols. Low VAF was defined as three standard deviations below the mean VAF in LB/B variants (35.1%). Frequencies of low VAF in LP/P and LB/B were used to calculate likelihood ratios (LR) and quantitative Bayesian ACMG evidence points for pathogenicity.

Results:

Low VAF in rare *NF1* variants were observed in 1.6% (714/43,457) of the patients, indicating that it is a common finding in germline genetic testing. Mean VAFs differed significantly among the classification groups: LP/P 41.1%, VUS 46.8%, and LB/B 47.4% (T-test $p < 0.0001$, compared to LB/B). Low VAF was more likely to be detected with LP/P variants (23.1%, 203/880), compared to LB/B variants (0.8%, 301/36,033) (Fisher's exact test $p < 0.0001$). Overall, patients with *NF1* LP/P variants were younger (mean 44.1 years) than those with LB/B variants (53.0 years) (T-test $p < 0.0001$) because patients with a clinical

diagnosis or suspicion of NF1 are more likely tested at a younger age. However, among those with low-VAF *NF1* variants, patients with LP/P variants were older (61.1 years) than those with LB/B variants (44.1 years) (T-test $p < 0.0001$), supporting the role of age-related clonal hematopoiesis.

In total, 4,145 unique rare *NF1* variants were identified. Low VAF was observed in 33.1% (128/387) of LP/P, 8.4% (189/2,263) of VUS, and 8.4% (125/1,495) of LB/B variants. This yields an LR of 3.94 (0.331/0.084) for low VAF, which corresponds to supporting evidence of pathogenicity and approaching moderate (Bayesian evidence point 1.87). Multiple independent observations of low VAF for the same variant increased the evidence strength: two observations (LR = 15.5, moderate evidence, 3 points), three (LR = 61.2, strong evidence, 5 points), and five or more (LR > 949, exceeds very strong evidence, 8 points).

Conclusions:
Low VAF in *NF1* variants provides supporting evidence for pathogenicity, with the strength of evidence increasing with multiple independent observations. Clonal hematopoiesis, particularly in older individuals, is likely a key mechanism underlying low VAF observed with pathogenic variants, but technical artifacts and benign variants in mosaicism and clonal hematopoiesis must also be considered. The Bayesian ACMG framework enables quantitative integration of low VAF as patient-specific phenotype data (ACMG code PP4), improving interpretation of *NF1* variants. The approach demonstrated here for *NF1* may be applicable to other genes associated with clonal hematopoiesis.