Low Allele Fraction, High Stakes: Discerning Germline Mosaic *TP53* Variants from Clonal Hematopoiesis of Indeterminate Potential (CHIP)

Magan Trottier[1], Kristin Kohler[1], Cassidy Carraway[1], Jennifer Herrera-Mullar[1], Marcy E. Richardson[1]

1. Ambry Genetics, Aliso Viejo, CA, USA

Background

- Clonal hematopoiesis of indeterminate potential **(CHIP)** occurs when acquired somatic variants cause hematopoietic expansion in the absence of an overt hematologic malignancy.[1]
- Older age and exposure to chemotherapy or radiation therapy are risk factors for CHIP.[2]
- CHIP is associated with variants at low variant allele fraction **(VAF)** in genes such as *ASXL1*, *DNMT3A*, and *TET2*[1] whereas heterozygosity in these genes can cause distinctive childhood-onset syndromes.
- *TP53* variants with low VAF can also be seen with CHIP as well as with mosaic Li-Fraumeni syndrome **(LFS)**, which can be hard to discern without additional data.[3]

AIM: Evaluate clinical and variant-level data from a genetic testing laboratory to inform the distinction between mosaic LFS and CHIP

Methods

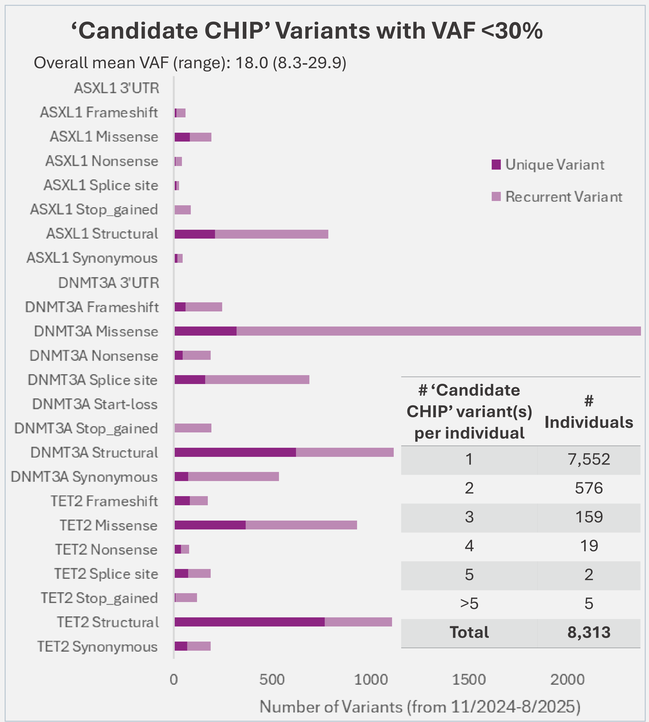
- Selected individuals tested from 11/2024 to 8/2025 at a single diagnostic laboratory with *TP53* likely pathogenic/pathogenic variants (**PVs**) and/or 'candidate CHIP' variants in the *ASXL1*, *DNMT3A*, or *TET2* genes with VAF <40% or <30%, respectively
- Identified which individuals had secondary confirmation testing for *TP53* (cultured fibroblasts)
- Excluded variants in the 'candidate CHIP' genes with allele frequency (**FAF**) ≥1% in gnomAD v4.1.0

LFS Group: Blood/saliva & cultured fibroblast testing identified same *TP53* PV

CHIP Group: *TP53* PV from blood/saliva testing was not identified in cultured fibroblast testing

Unknown Group: *TP53* PV identified in blood/saliva but confirmation testing not performed

Results



# 'Candidate CHIP' variant(s) per individual	# Individuals
1	7,552
2	576
3	159
4	19
5	2
>5	5
Total	**8,313**

Figure 1. Prevalence of variants in *ASXL1*, *DNMT3A*, or *TET2* with VAF <30% and FAF <1%. The inset table shows co-occurrence of these 'candidate CHIP' variants within individuals.

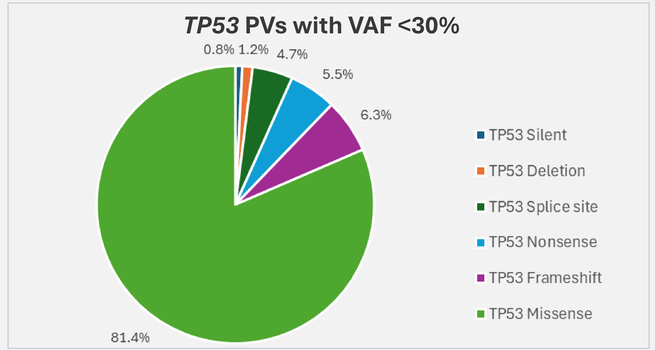


Figure 2. Prevalence of low VAF *TP53* PVs in this cohort tested from 11/2024-8/2025.

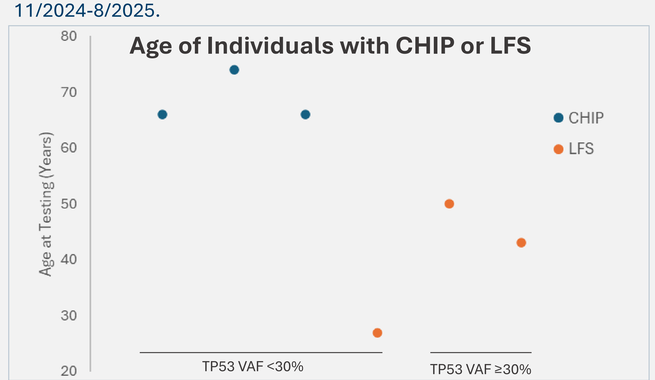


Figure 3. Age at testing for each individual with CHIP (n=3) or LFS (n=3). Not pictured above, average age at testing and average age at cancer diagnosis were similar for those with CHIP or LFS.

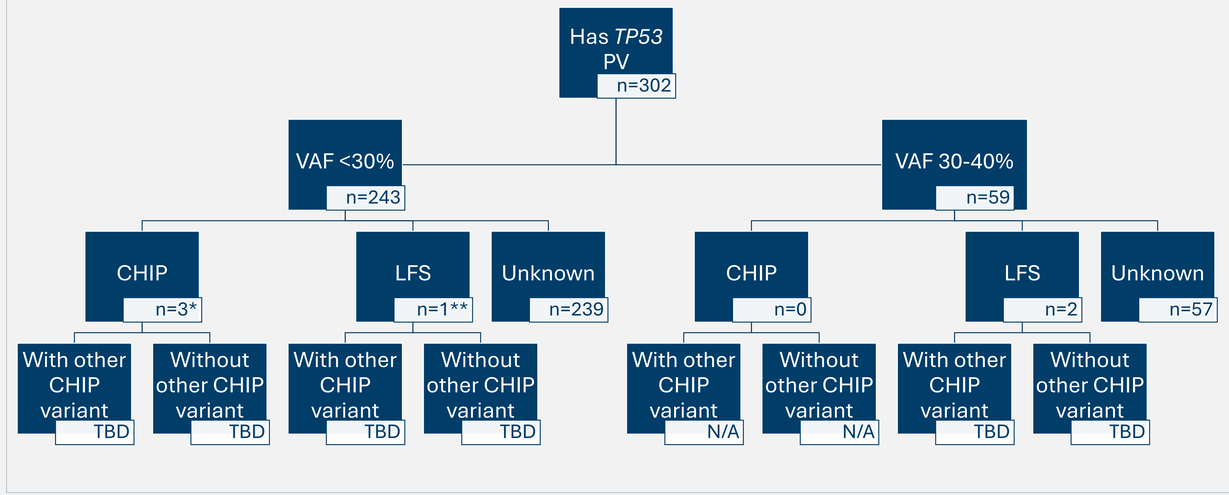


Figure 4. Summary of individuals with LFS or CHIP with VAF <40% or 30%, respectively, tested from 11/2024-8/2025. TBD = to be determined with additional analyses. *VAF for CHIP group were 11.7, 13.1, and 15-25%. **VAF for LFS group was 16.2%

Take Home Points

- Data suggest that individuals with a *TP53* LP/PV may be stratified into presumed CHIP based on VAF <30%, or possibly ≤15%, versus LFS based on VAF ≥30%, or possibly >15%. Age may help further stratify.
- Areas for future research include expanding this cohort and including all low VAF 'candidate CHIP' variants, stratifying based on co-occurring 'candidate CHIP' variant(s), controlling for prior treatments that may cause CHIP, and assessing genes beyond *ASXL1*, *DNMT3A*, and *TET2*.
- Our baseline data on unique and recurrent variants in *ASXL1*, *DNMT3A*, and *TET2* will help prioritize future evaluation of low VAF and co-occurring 'candidate CHIP' variants.

References

1. Genovese G, Kähler AK, Handsaker RE, *et al*. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014. PMID: 25426838.
2. Marshall CH, Arafa AT, Jaeger E, *et al*. Germline DNA Repair Gene Mutations and Clonal Hematopoiesis (CH) in 24,849 Patients with BRCA-Associated Cancers. *Cancers (Basel)*. 2025. PMID: 40361359.
3. Chao EC, Astbury C, Deignan JL, *et al*; ACMG Laboratory Quality Assurance Committee. Incidental detection of acquired variants in germline genetic and genomic testing: a points to consider statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2021. PMID: 33864022.