Low variant allele fraction as a predictor of *TP53* variant pathogenicity

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Approximately 30% of *TP53* variants identified in the blood of patients undergoing genetic testing for hereditary cancer are suspected to have somatic origin, mostly due to clonal hematopoiesis (CH), and this proportion increases with lower variant allele fraction (VAF). Positive selection has been proposed to be a mechanism driving CH, with somatic variants providing fitness advantage to a subset of blood cells. For this reason, we hypothesized that *TP53* variants implicated in CH on the basis of low VAF would be enriched for pathogenic variants. If true, low VAF status could be used a positive predictor of *TP53* variant pathogenicity. We compared VAF between 1069 *TP53* variants classified as (likely) pathogenic and 35327 *TP53* variants classified as (likely) benign using data from Ambry Genetics. VAF distribution was strikingly different between the groups: benign variants showed an expected normal distribution around 50:50 ratio (average 48.1, standard deviation 3.7), while pathogenic variants showed a bimodal distribution. We then compared the proportion of pathogenic and benign variants observed in different VAF bins, and determined that VAF <33.22 provided very strong evidence towards pathogenicity (likelihood ratio >350:1). Further, we found that this evidence type could be applied to approximately 5% of unique *TP53* germline variants of uncertain significance present in the same dataset. This data confirms that CH is a distinctive characteristic of pathogenic *TP53* variants, and provides a new interpretation of this phenomenon in that it can be used as a natural functional assay to assist with the interpretation of *TP53* germline variants.