Response to Holstege et al.

To the editor: After further analysis, we agree with the concerns of Holstege et al.[1] related to clonal hematopoiesis of indeterminant potential, or CHIP.[2] For our study,[3] we incorporated additional control data which resulted in younger control subjects. Age differences between case subjects and control subjects are acceptable for germline variation, but if age-associated variation such as CHIP contributes, it introduces a potential confound.

We previously viewed variants in the Integrated Genomics Viewer and by Sanger sequencing and qualitatively observed variation consistent with germline. In light of the possibility of CHIP raised by Holstege et al.,[1] we quantitatively reassessed allele balance, which revealed a bimodal distribution by variant type: loss-of-function (LoF) variants appear to be consistent with CHIP (median allele balance [AB] = 0.25), while non-LoF variants appear germline (median AB = 0.51). A similar bimodal distribution is present in the ADSP genomes replication cohort (median LoF AB = 0.20, median non-LoF AB = 0.44). We were only able to detect this effect when assessing allele balance by variant sub-grouping.

Holstege et al.[1] correctly point out the main interpretative issue: CHIP could lead to an overrepresentation of rare variation in *TET2* due to age stratification between case and control subjects. In addition, even if age is not sufficient to explain the observed enrichment, interpretative implications remain, i.e., disease risk could result in part from CHIP (independent of age effect). We therefore address two main points.

TET2 Association Remains after Adjustment for Age

Although nominal significance remains, the association is no longer genome-wide significant (SKAT discovery p = 0.0024, replication p = 0.015, combined p = 0.0016, all calculated as in the original paper with the addition of adjustment for age). Lack of genome-wide significance is a limitation, but we note that an association remains in replication cohorts (driven by ADSP genomes, p = 0.016). In addition, applying an age adjustment, while necessary to address the possible contribution of CHIP, might over-adjust any association not driven by CHIP because age is correlated with phenotype.

Association Remains when Analysis Is Constrained by Variation Type

We set a threshold for possible CHIP at the 5th percentile of all qualifying variation (AB = 0.32). An age adjustment is unnecessary for likely germline variation (AB > 0.32), which still exhibits nominal significance (SKAT discovery p = 2.2×10^{-4}, OR [±95% CI] = 19 [3–811]; replication p = 0.031, OR [±95% CI] = 1.7 [1.0–2.9]; combined p = 0.0497, OR [±95% CI] = 2.3 [1.4–3.8]). Therefore, germline variation (the majority of identified variants) remains a candidate for association with risk for neurodegeneration. For variants with AB < 0.32, we matched the age of the cohort (by pruning young control subjects until the control median matched the case subjects) and also adjusted for age. With this strict age matching and adjustment, there was an association through meta-analysis across UCSF and ADSP cohorts (SKAT p = 0.048, OR [±95% CI] = 2.3 [1.1–5.3]).

This new information suggests there may be yet unknown, age-independent contributions of *TET2*-driven CHIP to neurodegenerative disease. Previously shown as a strong risk factor for cardiovascular disease and stroke[4,5] and present in approximately 10% of individuals over 65,[6,7] comprehensive assessment and replication is now required to fully assess the role of CHIP in neurodegeneration.

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