The Role of Age-Related Clonal Hematopoiesis in Genetic Sequencing Studies

To the editor: With great interest we read the study of Cochran et al. in which they report significantly higher frequencies of rare loss-of-function variants in *TET2* in individuals with Alzheimer disease (AD) or frontotemporal dementia (FTD) as compared to healthy individuals.[1] As genotyping was performed by sequencing blood-derived DNA obtained from predominantly elderly individuals, we would like to discuss these results in view of recent developments in age-related clonal hematopoiesis (ARCH) research,[2,3] which is also known as clonal hematopoiesis of indeterminate potential (CHIP).

Hematopoietic stem cells (HSCs) continuously accumulate somatic mutations during aging.[4] ARCH arises when an HSC acquires a somatic mutation that confers a competitive survival advantage leading to its gradual expansion. With this competitive advantage, the mutated HSC will progressively increase its contribution to peripheral blood production. When the HSC "clone" contributes to a high enough fraction of peripheral blood cells, the somatic mutations it has acquired over time become detectable by conventional protocols for next-generation sequencing. We previously reported that at extreme ages (111 and 115 years), up to ~70%–75% of all peripheral blood cells were generated by one HSC clone and its subclones.[5,6]

ARCH is typically instigated by somatic mutations in genes previously linked with myeloid neoplasms, most notably in genes encoding the epigenetic regulators *TET2, DNMT3A*, and *ASXL1*.[7–10] In particular for somatic mutations in *TET2*, a strong correlation between chronological age and detectable carriership has been reported, of which a considerable part can be characterized as rare loss-of-function mutations.[7,8] Hence, given the nature of the study sample used by Cochran et al.,[1] i.e., blood tissue obtained from elderly individuals, it is reassuring, though not surprising, that the authors identified considerable numbers of rare loss-of-function mutations in *TET2*.

In light of the potential somatic origin of *TET2* mutations, our concern was raised when we noted the disparity in age between the cohorts contrasted by Cochran et al.[1] for the discovery phase of their study. The authors compared relatively old individuals with AD and FTD (median age 59 and 65 years) with younger healthy individuals (median age 40 years) and find that mutations in *TET2* associate with a 29-fold increased risk of AD/FTD (OR: 28.9, 95%CI 4.5–1,200, $p = 4.9 \times 10^{-7}$; AD subjects: n = 227; FTD subjects: n = 208; healthy control subjects n = 671). In comparison, a previous large-scale sequencing study conducted by Jaiswal et al.[7] reported that detectable somatic mutations in *TET2*, *DNMT3A*, and *ASXL1* were extremely rare in the blood of individuals aged <40 years, while nearly 10% of the individuals aged between 60 and 69 years were reported to carry detectable somatic mutations in these genes. In accordance, in the replication analysis conducted by Cochran et al.,[1] in which the age of the individuals with AD and FTD (median age 79 and 76 years) more closely resembled the age of healthy control subjects (median age 74 years), the authors reported only a 1.7-fold increased AD/FTD risk (OR = 1.7, 95%CI 1.2–2.6, $p = 6.1 \times 10^{-3}$; AD subjects n = 2,530; FTD subjects n = 319; healthy control subjects n = 2,457). Hence, the previously reported age-associated increase of carriers with somatic mutations in *TET2* could imply that the reported AD association in the discovery phase reported by Cochran et al.[1] was confounded by the differences in age between the AD/FTD subjects and the healthy individuals.

To conclude, we advocate utmost caution when interpreting the results of sequencing data assayed on the blood of elderly individuals, especially when considering rare mutations in genes previously linked to myeloid clonal expansions, such as *TET2*, *DNMT3A*, and *ASXL1*. In such events, it would be prudent to provide additional evidence supporting either the somatic or germline nature of these variants, to substantiate any observed association with age or disease.

Henne Holstege,[1,2,3,*] Marc Hulsman,[1,2,3]
Sven J. van der Lee,[1,2,3] and Erik B. van den Akker[3,4,5]
[1]Department of Clinical Genetics, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, the Netherlands; [2]Alzheimer Center Amsterdam, Department of Neurology, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, the Netherlands; [3]Pattern Recognition & Bioinformatics, Delft University of Technology, Delft 2628CD, the Netherlands; [4]Leiden Computational Biology Center, Leiden University Medical Center, Leiden 2300RC, the Netherlands; [5]Section of Molecular Epidemiology, Leiden University Medical Center, Leiden 2300RC, the Netherlands

*Correspondence: h.holstege@amsterdamumc.nl

References

1. Cochran, J.N., Geier, E.G., Bonham, L.W., Newberry, J.S., Amaral, M.D., Thompson, M.L., Lasseigne, B.N., Karydas, A.M., Roberson, E.D., Cooper, G.M., et al.; Alzheimer's Disease Neuroimaging Initiative (2020). Non-coding and Loss-of-Function Coding Variants in TET2 are Associated with Multiple Neurodegenerative Diseases. Am. J. Hum. Genet. *106*, 632–645.
2. Shlush, L.I. (2018). Age-related clonal hematopoiesis. Blood *131*, 496–504.
3. Steensma, D.P., Bejar, R., Jaiswal, S., Lindsley, R.C., Sekeres, M.A., Hasserjian, R.P., and Ebert, B.L. (2015). Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood *126*, 9–16.

4. Busque, L., Patel, J.P., Figueroa, M.E., Vasanthakumar, A., Provost, S., Hamilou, Z., Mollica, L., Li, J., Viale, A., Heguy, A., et al. (2012). Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. Nat. Genet. *44*, 1179–1181.
5. Holstege, H., Pfeiffer, W., Sie, D., Hulsman, M., Nicholas, T.J., Lee, C.C., Ross, T., Lin, J., Miller, M.A., Ylstra, B., et al. (2014). Somatic mutations found in the healthy blood compartment of a 115-yr-old woman demonstrate oligoclonal hematopoiesis. Genome Res. *24*, 733–742.
6. van den Akker, E.B., Makrodimitris, S., Hulsman, M., Brugman, M.H., Nikolic, T., Bradley, T., Waisfisz, Q., Baas, F., Jakobs, M.E., de Jong, D., et al. (2020). Dynamics of clonal hematopoiesis in a supercentenarian. BioRxiv. https://doi.org/10.1101/788752.
7. Jaiswal, S., Fontanillas, P., Flannick, J., Manning, A., Grauman, P.V., Mar, B.G., Lindsley, R.C., Mermel, C.H., Burtt, N., Chavez, A., et al. (2014). Age-related clonal hematopoiesis associated with adverse outcomes. N. Engl. J. Med. *371*, 2488–2498.
8. Genovese, G., Kähler, A.K., Handsaker, R.E., Lindberg, J., Rose, S.A., Bakhoum, S.F., Chambert, K., Mick, E., Neale, B.M., Fromer, M., et al. (2014). Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N. Engl. J. Med. *371*, 2477–2487.
9. Xie, M., Lu, C., Wang, J., McLellan, M.D., Johnson, K.J., Wendl, M.C., McMichael, J.F., Schmidt, H.K., Yellapantula, V., Miller, C.A., et al. (2014). Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat. Med. *20*, 1472–1478.
10. van den Akker, E.B., Pitts, S.J., Deelen, J., Moed, M.H., Potluri, S., van Rooij, J., Suchiman, H.E.D., Lakenberg, N., de Dijcker, W.J., Uitterlinden, A.G., et al.; Genome of The Netherlands Consortium (2016). Uncompromised 10-year survival of oldest old carrying somatic mutations in DNMT3A and TET2. Blood *127*, 1512–1515.

https://doi.org/10.1016/j.ajhg.2020.07.011.