

Comment on Hansen et al, page 261, and Fabre et al, page 269

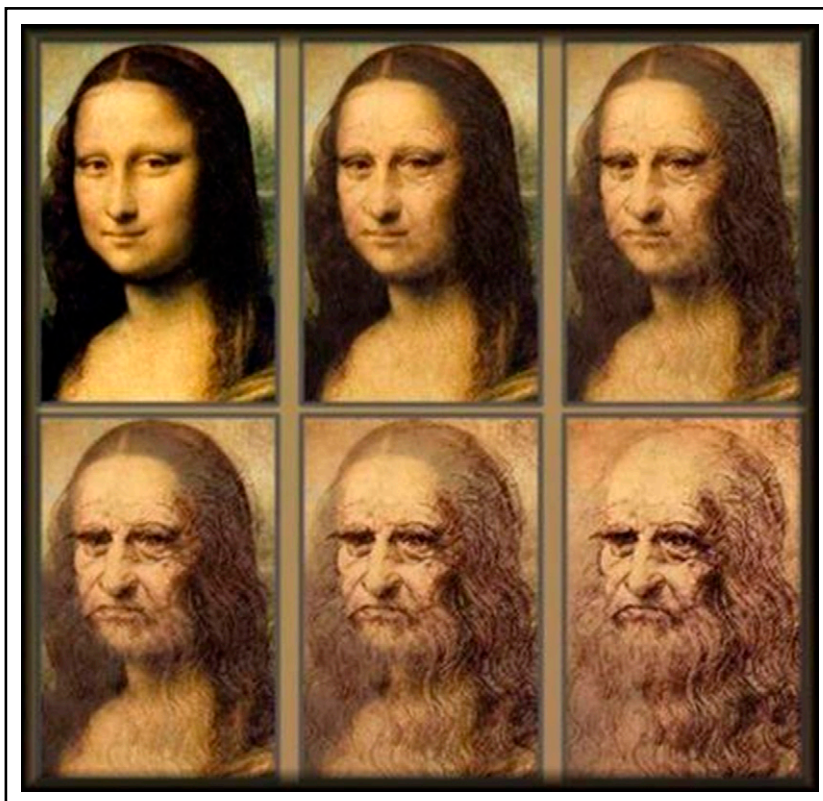
# Clonal hematopoiesis sees Twin Peaks

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**Clonal hematopoiesis (CH) is a fundamental process in the aging of the blood system; however, it remains enigmatic why some individuals of the same age carry mutations, whereas others do not or carry different mutations with different variant allele frequencies (VAF). In this issue of *Blood*, Fabre et al<sup>1</sup> and Hansen et al<sup>2</sup> address the differential roles of nature (genotype/heritability) vs nurture (environment) in the evolution of age-related clonal hematopoiesis (ARCH).<sup>3</sup> In these studies, twin concordance analysis was used to determine the germline contribution to ARCH.<sup>4</sup> In both studies, no hereditary component of ARCH could be detected. Therefore, it is most likely that the variability of ARCH in the population can be explained by differential environmental exposures. Although such a conclusion is reasonable, a few specific aspects of the nature vs nurture dilemma in CH need to be considered.**

It is important to understand that the problem of nature vs nurture does not apply to a particular individual. In any given person, the ARCH phenotype always emerges from the interaction of genotype

and the environment (see figure).<sup>5</sup> However, at the population level, ARCH is highly variable, as are many other quantitative traits. Likewise, ARCH variability in the population can be measured based



Age-related phenotypic heterogeneity with the same genetic background (dizygotic twins).

on multiple parameters: VAF, number of mutations, type of mutations, age of first appearance, and more. The differential contribution of nature vs nurture can explain such phenotypic variability. The facts that ARCH is common in the elderly population and has similar concordance levels in both monozygotic and dizygotic twins suggest that the germline determinants of ARCH are relatively common in the general population and probably have little impact. That the germline players are common may explain why dizygotic twins can share the phenotype at the same level as monozygotic twins, given they were exposed to the appropriate environment. This is similar to the situation in scurvy. All humans (common genetic variant) do not carry the genetic determinants to produce vitamin C. Therefore, once a vitamin C-deficient environment occurs, scurvy develops. However, the disease that develops differs in severity, depending on, for example, the length of the vitamin C deficiency. This analogy suggests that common genetic variants with a weak contribution to the phenotype might still exist and their identification is going to be a major challenge because they are often ignored in the conventional genome-wide association studies (GWASs).<sup>6</sup> Furthermore, because the contribution of these hypothetical common variants to the phenotype is weak, possibly polygenic, and is only manifest under specific environmental conditions, the experiments needed for functional proof are extremely complicated. Therefore, it is predicted that large GWASs on ARCH might be inconclusive, and it will be even harder to follow their predictions by functional studies.

ARCH is a condition that increases the risk for myeloid malignancies<sup>7,8</sup> and cardiovascular diseases.<sup>9</sup> Like many other conditions in medicine, ARCH probably (1) Is complex (has different etiologies); (2) Is a quantitative trait; (3) Has some hereditary polygenic contribution to its pathogenesis in different populations. Therefore, let us examine each of these points. The current twin studies grouped all subtypes of ARCH together, but also studied separately different mutation subtypes like *DNMT3a* and *TET2* (2) and could not identify hereditary predisposition in any of these subgroups. Although this is a valid and important approach, it is limited to the most frequently mutated genes because the studies were not powered for the less common genes. Therefore, one cannot exclude that ARCH due to mutations in

the spliceosome (rare phenotype) might have some hereditary component.

ARCH is a quantitative trait much like blood pressure and height. Although ARCH has been defined so far as a binary attribute, the true nature of ARCH is described by the clonal diversity of the blood system. Genetic diversity is defined by the number of hematopoietic stem and progenitor cells in a single individual and their relative contribution to the mature blood pool. This genetic diversity can change with aging (ARCH) but also due to other causes (aplastic anemia, chemotherapy, etc).<sup>3</sup> Accordingly, one can estimate the hereditary contribution to the blood system genetic diversity (as a continuous measure). Because this parameter changes with age and probably with sex and other parameters, the study design should take all these potential contributors into account. For example, ARCH or genetic diversity at a young age might be correlated with a stronger genetic component.

In some cases, a phenotype can have a stronger genetic predisposition in a specific subpopulation. A good example is end-stage kidney disease in African Americans.<sup>10</sup> ARCH has not been well characterized, yet in many human subpopulations, such epidemiological studies could shed light on specific genetic predisposition.

Another interesting aspect that was observed in both studies is the fact that in few cases the preleukemic mutation was acquired in utero (both twins carried the same mutation). This observation again stresses the importance of the environment in the evolution of ARCH. Only after many years did the ARCH phenotype evolve despite the presence of the mutation in hematopoietic stem cells for at least 50 years (in some of the cases). In utero acquisition of preleukemic mutations might be even more common but could be missed because of the discordance of the ARCH phenotype.

Altogether, the important conclusions from these twin studies suggest that, among the elderly, CH is more strongly influenced by the environment, with the caveats described above. These studies suggest that the search for environmental contributors to ARCH is more pressing than genetic predispositions.

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## MYELOID NEOPLASIA

Comment on Bridgford et al, page 287

# MPL membrane domain sequencing goes deep

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**In this issue of *Blood*, Bridgford et al<sup>1</sup> present the first systematic approach to uncover all possible activating single point mutations within and around the transmembrane domain (TMD) of the thrombopoietin receptor (TpoR; also known as MPL). Several canonical activating MPL mutations (S505N and W515K/L/A/R) have been discovered through structure-function studies and patient sequencing,<sup>2,6</sup> but there remain “triple-negative” essential thrombocythemia and primary and secondary myelofibrosis cases, as well as hereditary thrombocytosis cases, without established driver mutations (ie, no JAK2, CALR, or MPL variants). The TpoR is a cytokine receptor that can be easily activated by dimerization in several different orientations,<sup>7,8</sup> which is unusual, but can explain the multitude of mutations that lead to an active or partially active phenotype. However, which specific variants can activate the receptor and drive disease?**

Bridgford et al address this question with deep mutational scanning to evaluate all possible single amino acid substitutions in the human TpoR TMD for their ability to confer cytokine-independent growth in Ba/F3 cells. The authors undertook an incredibly efficient examination of all possible 19 amino acid residues (saturation mutagenesis) at each of the 29 positions of the target (TpoR) juxtamembrane-transmembrane region (residues 488 to 516). In all, 580 mutants were screened

(29 positions, 20 codons each including the stop codon) representing the 19 remaining amino acids at each position. An enrichment was detected for the classical S505N and W515 mutations, but also for novel mutations (S493C/L, L498W, V501S, L502S/C, L508Q) (see Figure 1A in Bridgford et al).

This strategy was also used to answer another important question: whether the constitutive signaling of a canonical MPL